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EPIDEMIOLOGICAL, CLINICAL AND PATHOGENETIC STUDIES OF ACUTE INTERMITTENT PORPHYRIA

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av

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Epidemiological, clinical and pathogenetic studies of acute intermittent porphyria

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ABSTRACT: Porphyrias are inherited metabolic disorders characterised by an impairment of heme biosynthesis. Acute intermittent porphyria (AIP) is the most common of the acute porphyrias in Sweden. Acute attacks of AIP are characterised by neuro-psychiatric symptoms, including epileptic seizures. Environmental and acquired factors are related to the induction of symptoms. Acute attacks of AIP are treated with high doses of glucose and/or hematin infusions.

The pathogenesis of the neuro-psychiatric symptoms is not known. Reversible white-matter lesions, probably due to vasospasm, have been seen on brain MRI. Similarities between multiple sclerosis (MS) and AIP have previously been described, but to our knowledge no study has investigated whether AIP-gene carriers have white-matter lesions seen on brain MRI or oligoclonal bands (OB) in cerebrospinal fluid (CSF).
The percentage of AIP-gene carriers who have experienced epileptic seizures has been calculated at 10-20%, but previous investigations are derived from highly selected clinic-based studies. Studies were therefore undertaken to investigate the prevalence of epileptic seizures, the relationship of seizures to AIP, the type of seizures and the relationship of seizures to other factors such as melatonin.

A case report described the disappearance of porphyric attacks after the onset of diabetes mellitus (DM). In our study, we investigated the rate of attacks after the onset of DM. For many years, clinical issues relating to AIP have not been a focal area. We therefore carried out a study to update our knowledge of the clinical course of AIP in order to improve prevention, control and treatment. In our studies of AIP-gene carriers and epileptic seizures, we found that epileptic seizures are less common than has previously been described (3.7%) and they are not very different from what is expected in the general population, but the prevalence of 5.1% of seizures with manifest AIP is higher than in the general population. The seizures may be generalised or partial and the seizure frequency was generally low. The AIP-gene carriers who had had epileptic seizures had a lower melatonin excretion level in their urine compared with gender- and aged-matched AIP-gene carriers' relatives without epileptic seizures, which may indicate that melatonin plays a possible anti-convulsive role.

In our study of AIP and DM, no subject had an attack of AIP after the onset of DM. White-matter lesions on brain MRI were seen in 25% of the AIP-gene carriers examined outside attacks. One carrier had elevated protein levels in the CSF, but no carrier had cells or OB in the CSF.

In our population-based study, 356 DNA-confirmed AIP-gene carriers from northern Sweden participated. Manifest AIP (MAIP) was identified in 42%, 65% of whom were women. Eight mutations were found. Women were more severely stricken by AIP attacks in terms of number and duration, hospital admission and early onset. Men (30%) reported most attacks > 40 years of age. The most commonly reported symptoms during attacks were severe abdominal pain (86%), fatigue (42%), constipation (41%), vomiting (36%), muscle pain (30%), psychiatric symptoms (29%), pareses (20%) and sensory impairment (10%). Chronic AIP symptoms were reported by 18%. Precipitating factors were often reported: menstruation (31%), psychological strain (30%), certain drugs and fasting (20%), infection and alcohol (14%), physical strain (12%) and pregnancy (5%). Smoking was more frequent in MAIP and was associated with the number of AIP attacks. Some 30% of MAIP carriers used drugs that were not considered safe (in 1999), mainly diuretics, calcium antagonists and ACE inhibitors. Twenty per cent of MAIP carriers reported that they were receiving a disability pension due to AIP. Elevated levels of ASAT, bile acids, creatinine, creatinine clearance, U-ALA and U-PBG were often found in MAIP-gene carriers. Hypertension, renal impairment and pain in the legs were associated with MAIP. Hepatoma was strikingly over-represented.To summarise; epileptic seizures are less common than has previously been described, melatonin may have an anti-convulsive effect and DM may have a beneficial effect on MAIP-gene carriers. White-matter lesions are seen on brain MRI. The lesions are unspecific but may relate to the patients' porphyria. AIP is not a harmless disease. A large percentage of the AIP-gene carriers had frequent attacks, severe symptoms, long-lasting fatigue and chronic AIP and women were more severely stricken. Effects on the kidneys, blood pressure and the liver, including HCC, were evident. Measures should be taken to improve the quality of life and prognosis for AIP-gene carriers.

Key words: acute intermittent porphyria; seizures; melatonin; diabetes mellitus; white-matter lesions; symptoms; attack; prognosis; Sweden.

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Ingemar Bylesjö

Medical dissertation

Family Medicine
Department of Public Health and Clinical Medicine
Umeå University 2008

ABSTRACT

Porphyrias are inherited metabolic disorders characterised by an impairment of heme biosynthesis. Acute intermittent porphyria (AIP) is the most common of the acute porphyrias in Sweden. Acute attacks of AIP are characterised by neuro-psychiatric symptoms, including epileptic seizures. Environmental and acquired factors are related to the induction of symptoms. Acute attacks of AIP are treated with high doses of glucose and/or hematin infusions.

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A case report described the disappearance of porphyric attacks after the onset of diabetes mellitus (DM). In our study, we investigated the rate of attacks after the onset of DM. For many years, clinical issues relating to AIP have not been a focal area. We therefore carried out a study to update our knowledge of the clinical course of AIP in order to improve prevention, control and treatment. In our studies of AIP-gene carriers and epileptic seizures, we found that epileptic seizures are less common than has previously been described (3.7%) and they are not very different from what is expected in the general population, but the prevalence of 5.1% of seizures with manifest AIP is higher than in the general population. The seizures may be generalised or partial and the seizure frequency was generally low. The AIP-gene carriers who had had epileptic seizures had a lower melatonin excretion level in their urine compared with gender- and aged-matched AIP-gene carriers' relatives without epileptic seizures, which may indicate that melatonin plays a possible anti-convulsive role.

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SAMMANFATTNING (in Swedish)

Porfyrier är ärftliga metaboliska sjukdomar som beror på enzymdefekter i hemesyntesen. Akut intermittent porfyri (AIP) är den vanligaste av de akuta porfyrierna i Sverige på grund att många anlagsbärare finns i norra Sverige. Akuta attacker av AIP ger neurologiska-psykiatriska symptom och utlöses oftast av miljöfaktorer som läkemedel, fasta, rökning, alkohol, narkos etc men även av endogena faktorer som menstruation. Kvinnor drabbas oftare av attacker än män och cirka 40% av anlagsbärarna har i norra Sverige drabbas av akuta attacker, manifest AIP (MAIP). De vanligaste symptomen vid attack är buksmärtor, muskelsvaghet, kräkningar, förstoppning, muskelsmärtor, högt blodtryck, snabb hjärtrytm, psykiska symptom men även epileptiska kramper, förlamning i armar och ben samt andningsförlamning kan förekomma. Orsaken till sjukdomssymptomen är inte känd men symptomen kommer från de olika nervsystemen.

Målsättningen med studierna var att undersöka hur vanligt det var med epileptiska kramper, om melatonin skulle kunna användas mot epileptiska kramper, om en diabetisk metabolism hade någon påverkan på AIP symptom, om det fanns förändringar i hjänan och spinalvätskan liknade dem man ser vid mutilpe sclerosis (MS), samt i en studie innefattade alla AIP anlagsbärare i norra Sverige beskriva klinisk bild, utlösande orsaker, medicinska konsekvenser-komplikationer, kroniska besvär, association till andra sjukdomar, sjukhusvård, antal porfyriattacker, ålder vid första attack, rökning/attacker, laboratorieprover etc.

Epilepiska kramper sägs inte vara”ovanligt” bland AIP anlagsbärare, 10-20% anges i olika studier. Vi fann att det var mindre vanligt med epileptiska kramper än som tidigare publicerats (3.7%), men vanligare än vad som skulle kunna förväntas i den allmänna befolkningen. De AIP anlagsbärare som hade haft kramper hade lägre utsöndring av melatonin i urinen jämfört med släktingar med AIP som inte hade haft kramper, vilket indikerar att melatonin kan ha en anti-kramp effekt.

Melatonin har i djur- och humanstudier visat sig ha en effekt mot kramper. Vi ville undersöka om de som hade haft kramper hade en lägre nivå av melatonin som en möjlig orsak till kramperna samt eventuellt använda melatonin mot kramperna då de flesta anti-kramp läkemedel kan ge porfyri attacker.

Socker används som behandling vid akut attack. En fallrapport har visat att attacker av AIP försvann efter diabetes diagnos. I vår studie fann vi att ingen av de 16 AIP anlagsbärarna hade några AIP symptom efter diabetes diagnos.

Fallrapporter har pekat på likheter mellan AIP och MS. Vi undersökte hjänan med MR och spinalvätskan på16 AIP anlagsbärare. Inga bärare hade oligoklonala band i spinalvätskan (ca 90% av MS patienterna har det). En av 4 AIP anlagsbärare hade vitsubstans förändringar på hjärnan.

Kliniska data på AIP har inte publiserats på många år. Vi undersökte 356 vuxna AIP anlagsbärare från de 4 nordligaste länen i Sverige. Vi fann att 42%, 65% av dessa var kvinnor hade haft symptom (MAIP). Åtta olika mutationer hittades. Kvinnorna drabbades hårdare i form av fler och längre attacker och behövde oftare sjukhusvård. Män rapporterade flest attacker >40 år. Vanligaste symptom under attack var buksmärtor (86%), trötthet (42%), förstoppning (41%). Kroniska besvär rapporterades av 18%. Utlösande faktorer: Menstruation (31%), psykologisk stress (30%), vissa läkemedel och fasta (20%), infektion och alkohol (14%), fysisk stress (12%), graviditet (5%). Rökning var associerad till många attacker. 30% av de med MAIP använde läkemedel som inte bedömdes som säkra (1999). 20% av de med MAIP attacker rapporterade att de hade förtidspension pga AIP. Förhöjda nivåer av ASAT, gall-syror, kreatinin, kreatinin-clearance, urin-aminolevulinic syra och porfobilinogen sågs oftare hos de med MAIP. Högt blodtryck, njurpåverkan och smärtor i benen var associerade till MAIP. Vi fann en hög frekvens av primär lever cancer.

Sammanfattning: Epileptiska kramper är ovanligare än tidigare beskrivits. Melatonin kan möjligen ha en anti-kramp effekt, och diabetes kan vara positivt för de med MAIP. Vit-substans förändringar som fanns på MR av hjärnan , är ospecifika men kan vara relaterade till AIP. AIP är inte en harmlös sjukdom. Många AIP gen-bärarna hade frekventa attacker, allvarliga symptom, långvarig trötthet, och kroniska besvär. Kvinnor var hårdast drabbade. Man fann effekter på njurar, blodtryck och lever inklusive primär lever cancer. Åtgärder bör vidtagas för att öka livskvaliteten och prognosen för gen-bärare med AIP.

“grey spirit yearning in desire
to follow knowledge like a sinking star
beyond the utmost bound of human thought”

“grå ande som trängtar i begär
att följa kunskapen som en sjunkande stjärna
bortom den mänskliga tankens yttersta gräns”

Lord Tennyson

To Elisabeth

Contents

LIST OF PUBLICATIONS

The thesis is based on the following orginal papers, which will be referred to by their Roman numerals:

I. **Bylesjö I,** Forsgren L, Lithner F, Boman K. Epidemiology and clinical characteristics of seizures in patients with acute intermittent porphyria. Epilepsia 1996; 37: 230-35.

II. **Bylesjö I,** Forsgren L, Wetterberg L**.** Melatonin and epileptic seizures in patients with acute intermittent porphyria. Epileptic Disorders 2000; 2: 203-8.

III. Andersson C, **Bylesjö I,** Lithner F. Effects of diabetes mellitus on patients with acute intermittent porphyria. Journal of Internal Medicine 1999; 245: 193-7.

IV. **Bylesjö I,** Brekke O-L, Prytz J, Skjeflo T, Salvesen R. Brain Magnetic Resonance Imaging white-matter lesions and cerebrospinal fluid findings in patients with acute intermittent porphyria. European Neurology 2004; 5: 1-5.

V **Bylesjö I,** Wikberg A, Andersson C. Clinical aspects on acute intermittent porphyria in northern Sweden: a population-based study. Submitted.

Abbreviations

AEDs	Anti-epileptic drugs
AIP	Acute intermittent porphyria
ALA	5-aminolevulinic acid
ALAS	5-aminolevulinic acid synthase
BBB	Blood brain barrier
CRIM	Cross-reactive immunological material
CSF	Cerebrospinal fluid
DM	Diabetes mellitus
EEG	Electroencephalography
GABA	Gamma-aminobutyric acid
HCC	Hepatocellular carcinoma
HMBS	Hydroxymethylbilane synthase = PBGD
Latent AIP	Gene carriers with laboratory evidence of PBGD deficiency but no history of AIP symptoms
Manifest AIP	Gene carriers with laboratory evidence of PBGD deficiency and previous episodes of AIP symptoms
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NO	Nitric oxide
NOs	Nitric oxide synthase
OB	Oligoclonal band
PBG	Porphobiliogen
PBGD	Porphobilinogen deaminase = HMBS
5-HT	5-hydroxytryptamine (serotonin)
5-HIAA	5-hydroxy indole acetic acid

Introduction

The porphyrias are inherited metabolic disorders, characterised by impairments in heme biosynthesis [11]. Each disorder is caused by a partial deficiency in one of seven of the eight enzymes in the heme biosynthetic chain.

The porphyrias are classified as either hepatic or erythropoietic, depending on the principal site of expression of the enzymatic deficit.

In acute intermittent porphyria (AIP), a hepatic porphyria, the most common of the acute porphyrias diagnosed in Sweden and Norway, the gene for the third enzyme in the heme biosynthetic pathway, porphobilinogen deaminase (PBGD), is mutated.

According to Anderson *et al*., 80-90% of AIP-gene carriers are asymptomatic throughout their lives[11]. In studies from Finland and Sweden, however, 40-50% of carriers of the mutated gene had experienced symptoms of AIP [12, 50, 137].

Acute attacks of AIP may be life threatening. Mortality during an acute attack has been reported to be as high as 50-60% [256]. With modern treatment, however, an acute attack of porphyria is only rarely lethal in Scandinavia.

However, an American report found that the mortality rate was three times higher among patients with AIP as compared to the general population and the major cause of this increase in mortality was symptoms associated with the porphyric attack itself [129].

History

Stokvis [240] reported the first case of acute porphyria in 1889, a woman who died after taking sulphonal. Stokvis described “two peculiar urinary pigments” and identified one of them as “haematoporphyrin” but believed it had no connection with sulphonal. Two years later, Hammarsten [108, 109] and Salkowski [226] showed that sulphonal was actually the cause of the haemoporphyrinuria. Early clinical reports of acute porphyria came from Harley [113] in Britain; Geill [94], Fehr [83] and Fridenreich [90] in Denmark; Bresslauer [47] in Austria; Jolles [131], Kober [142] and Kast [135] in Germany and Evensen [81] in Norway. Günter, who regarded the disorder as an inborn error of metabolism, presented the first clinical review of different types of porphyria in 1925 [107]. The porphyrin nomenclature is based on the Nobel laureate Hans Fischer’s original work on heme chemistry from 1934. Hans Fischer stated: “Porphyrins, the compounds which make grass green and blood red” [86].

A considerable part of the first clinical and biochemical contributions on porphyria came from Sweden, e.g. Hammarsten [108-110], Hedin [115] and Westermark [262]. Later, in 1934, Waldenström described eleven cases of acute porphyria from the northern part of Sweden, while Beronius in 1935 [31] described five cases of acute porphyria in Skellefteå (also in northern Sweden). One year later, Einar Wallquist, a general practitioner in Arjeplog (in the western part of northern Sweden), was able to construct a family tree displaying eight generations dating back to 1701 [77].

In his thesis in 1937, Waldenström [255] reported chemical and clinical data from 103 cases from 19 families with acute porphyria. He proposed an autosomal dominant inheritance and

subsequently introduced the term “acute intermittent porphyria”. Waldenström isolated and identified uroporphobilin III from urine, a substance that was excreted during attacks of acute porphyria, and, together with Vahlquist, he introduced the name porphobilinogen (PBG) [258]. In a review in 1939 of the neurological symptoms of porphyria, Waldenström [256] found that most patients who died during an acute attack of porphyria had been treated with barbiturates. The avoidance of barbiturates and similar drugs has contributed to a drop in mortality during acute attacks from 50-60% to almost zero in Sweden.

In 1957, Waldenström introduced the hypothesis that different forms of porphyria were derived from defects in various enzymes necessary for the biosynthesis of heme [257]. Two years later, Rimington was able to describe in detail the biosynthesis of hemoglobin and, during the following decades, all the enzymes in the heme pathway were identified (see, for example, [74, 183]).

In 1967, Wetterberg investigated in more detail the psychiatric manifestations of AIP and also reported on certain clinical and social aspects of the disease. He postulated an association between AIP and mental illness [263].

Lannfelt developed an immunological method to measure the concentration of PBGD protein in erythrocytes in 1990 [153]. He found two genotypic variants of AIP in members of Swedish AIP families, one variant with reduced PBGD concentrationa and various reductions in the PBGD-specific activity (cross-reactive immunological material, CRIM, negative) and one variant with elevated PBGD concentrations and a 50% reduction in PBGD enzyme activity (CRIM-positive). The determination of PBGD concentration by an ELISA technique supplementing previous methods enabled the identification of additional asymptomatic AIP-gene carriers.

Lee *et al*. [156] identified the mutation in the PBGD gene in the families with AIP described by Waldenström and Wetterberg. The mutation was a guanine-adenine substitution in exon 10. The mutation changes the codon for Trp 198 to a stop codon.
Lundin *et al*. [165] made a more thorough investigation of genetic aspects, including the regulation of the PBGD gene. They found a considerable genetic heterogeneity in Swedish AIP-gene carriers.

Andersson [12] evaluated in clinical practice the mutation analysis and biochemical diagnostic methods and investigated the incidence of hepatocellular carcinoma, hypertension, renal lesions and psychological and social problems among AIP-gene carriers from the municipalities of Arjeplog and Arvidsjaur in northern Sweden.

Heme biosynthetic pathway

By associating different metals, porphyrins give rise to the "pigment of life", chlorophyll, heme and cobalamin. In eucaryotic cells, heme acts as a prosthetic group in a variety of compounds related to the storage and transport of oxygen, the protection of cells from free oxygen radicals, the transfer of electrons and the synthesis of ATP and cytochrome P450 which is involved in the de-oxification of various compounds and the metabolism of steroid hormones. Most of the heme in the body (80-90%) is produced in the bone marrow. In the liver, heme is produced rapidly in response to metabolic needs draining the free cellular heme pool [249].

Heme is synthesised by an array of eight enzymes [11, 219]; see Figure 1.

1. The initial mitochondrial enzyme, 5-aminolevulinic acid synthase (ALAS), catalyses the formation of 5-aminolevulinic acid (ALA) from succinyl-coenzyme A and glycine.
2. In the next step, two molecules of ALA form porphobilinogen (PBG), a reaction which is catalysed by the cytosolic enzyme, 5-aminolevulinate dehydratase.
3. In a further reaction, four PBG molecules are condensed by the cytosolic enzyme, hydroxymethylbilane synthase (HMBS), also named porphobilinogen deaminase (PBGD), to form a linear tetrapyrrol intermediate, the hydroxymethylbilane also called preuroporphyrinogen.
4. The linear tetrapyrrol is ring-closed by the cytosolic enzyme uroporphyrinogen III synthase to form uroporphyrinogen III (URO III).
5. The decarboxylation of URO III to coproporphyrinogen III (COPRO III) is catalysed by uroporphyrinogen decarboxylase in the cytosol.
6. The final three modifications to yield the end product, heme, take place in the mitochondria. Firstly, coproporphyrinogen oxidase converts COPRO III to protoporphyrinogen.
7. Protoporphyrinogen is converted to protoporphyrin IX by the enzyme protoporphrinogen oxidase.
8. In the final step, ferrous iron is inserted into protoporphyrin to form heme. The reaction is catalysed by ferrochelatase.

The first and the last three enzymes are located in the mitochondria, while the remainder are located in the cytoplasm.

Uroporphyrinogen, ALA and PBG are water soluble and are excreted in both urine and faeces. Coproporphyrinogen is excreted in both urine and faeces, but protoporphyrinogen is only excreted in faeces.

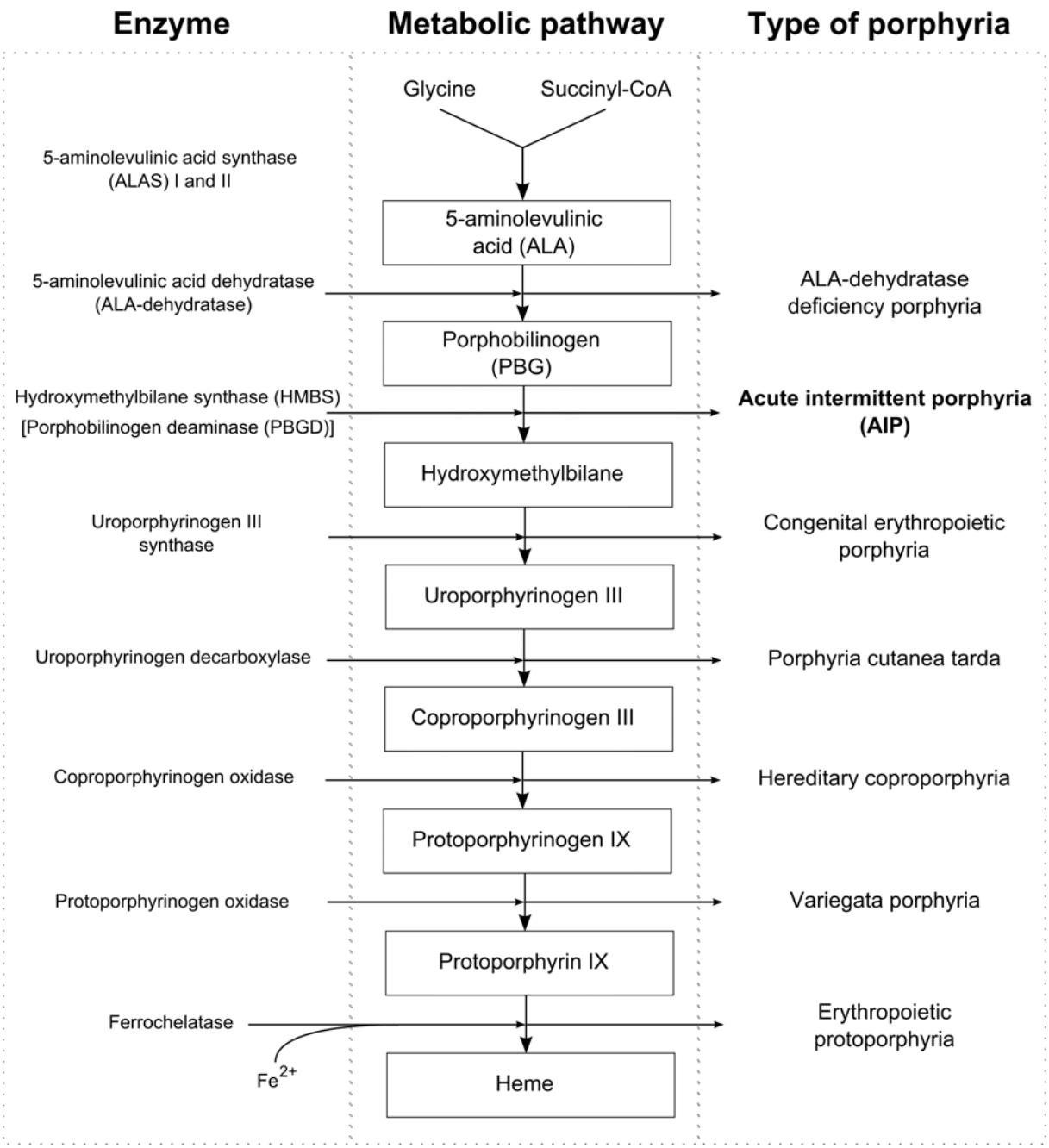

Figure 1. Heme biosynthesis and different porphyrias.

Regulation of heme synthesis

Heme biosynthesis is regulated by two tissue-specific ALAS isoenzymes, which are coded by two separate genes [37]. The majority of the total heme is synthesised in the erythroid cells (80-90%) in the bone marrow for incorporation into haemoglobin. The regulation of these syntheses involves ALAS-2 and is designed for the uninterrupted production of heme. On the other hand, the housekeeping ALAS gene, ALAS-1, is ubiquitously expressed in response to current metabolic needs due to the fact that all nucleated cells must synthesise heme for respiratory cythochromes. Heme is produced in the liver for various proteins, especially microsomal cytochrome P450, and heme represses the synthesis of ALAS-1 [89].

Acute intermittent porphyria

Genetics

Acute intermittent porphyria is caused by heterogeneous groups of mutations in the PBGD gene and AIP is inherited in an autosomal dominant manner with incomplete penetrance. The mutated enzyme PBGD is the third enzyme in the heme biosynthetic pathway. The activity of PBGD is approximately 50%, but the enzyme activity encoded by the normal allele is normally enough to maintain the normal demand for heme [101].

The human PBGD enzyme exists in two isoforms, i.e. a housekeeping enzyme with 361 amino acids and an erythrocyte-specific variant 17 amino acids shorter [102].
The PBGD gene has been mapped to chromosome 11q24.1-q24.2 [193, 259]. The genomic sequence spans 10 kb and consists of 15 exons with a total coding region of 1,100 bp [212]. The two different isoforms are produced by transcriptional initiation from two separate promotors [53, 211]. The non-erythroid promotor is positioned in front of exon 1 and, after splicing, exon 1 is connected to exons 3-15. All tissues, in contrast to the promotor 1 which is exclusively present in erythrocytes, use this promotor.

The first mutation in the PBGD gene was reported in 1989 [102]. To date, 246 mutations have been reported in the PBGD gene, also called the HMBS gene (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=HMBS). The most frequent mutation in the PBGD gene in Sweden and Norway, W198X, is located on exon 10 [156]. A subtype of AIP with normal PBGD activity in the erythrocytes was first described in Finland [187]. This variant has also been observed in Germany [104] and Sweden [88].

Diagnosis

Acute intermittent porphyria is diagnosed on the basis of genealogical data, clinical symptoms (if applicable) and according to standard and biochemical criteria including the analysis of U-ALA and U-PBG, erythrocyte PBGD and it is confirmed by DNA mutations in the PBGD gene.

Prevalence

Sweden has the highest prevalence of AIP in the world, about 10 per 100,000 inhabitants, due to the high prevalence of AIP in northern Sweden and especially in the two municipalities of Arjeplog (2%) and Arvidsjaur (0.5%) in the northern part of Sweden [12]. At the present time, 38 different mutations are known in Sweden. Floderus *et al*. [88] have published a review of the prevalence of carriers and mutations in the various regions of Sweden. About 1,000 AIP-gene carriers are diagnosed and registered at the National Centre of Porphyria, Stockholm, Sweden, and about half of them are living in the four northernmost counties of Sweden.

Until now, eight different mutations leading to AIP have been reported in Norway. The mutations are spread in the population all over the country (10 per 100,000) (Tjensvoll 2000 personal commication). A concentration of AIP-gene carriers (0.6%) is reported from the municipality of Saltdal (5,000 inhabitants), which borders on the municipality of Arjeplog in northern Sweden. During the 1980s, approximately 20 patients in Saltdal were found to have AIP. In 1999, 60 individuals with AIP had been identified in the county of Nordland (240,000 inhabitants, in which the municipality of Saltdal is included). All these persons have the AIP mutation (W198X), which is virtually the only mutation found in Arjeplog.

In Finland, the prevalence of gene carriers has been estimated at three per 100.000 [188]. This figure may well be an underestimation, as the prevalence among blood donors with low PBGD levels may be 1:500 donors to 1-1,500 [187].

In the USA, the frequency of AIP-gene carriers is estimated at five per 100,000 [11], while it is three per 100,000 in Western Australia [252].

Pathogenesis of the acute porphyric attack and precipitating factors

An acute attack of AIP is usually precipitated by environmental or acquired factors, such as drugs and impaired nutritional status. Barbiturates, sulphonamides, oral contraceptives, enzyme-inducing anticonvulsants and antidepressants are the drugs most commonly involved. Alcohol consumption, endogenous and exogenous sex hormones, infection and stress may also precipitate attacks [7, 11, 12, 15, 66, 184, 185, 256, 263]. Some women regularly have pre-menstrual attacks and pregnancy may provoke an exacerbation [171], but this has not been confirmed in recent studies [15, 122, 137].

The rate-limiting step in the biosynthesis of heme is the condensation of succinyl coenzyme A and glycine to form ALA [219], a reaction catalysed by the mitochondrial enzyme ALA-synthase (ALAS), EC 2.3,1.37. The formation of ALAS is controlled by the concentration of heme itself, through a negative feedback loop. The concentration of heme generated within the mitochondrion is insufficient to inhibit the enzyme and ALAS control is therefore thought to be exerted by a putative “free heme pool” in the cytosol [185].

In its biologically active form, heme is bound to various proteins to form heme proteins, which include haemoglobin, myoglobin and the cytochromes (including the P 450 series), along with enzymes involved in oxidation and hydroxylation reactions. If the need for heme is increased due to the administration of drugs, which induce cytochrome P 450, ALAS will increase rapidly [66, 184]. When there is a partial block in the synthetic pathway, the result may

be a heme deficiency that induces a compensatory increase in the activity of ALAS and, as a result, the production of ALA in the liver will be increased. In the face of a PBGD deficiency, there is an accumulation of its substrate PBG. Consequently, there will be an accumulation of ALA and PBG during attacks of AIP.

The mechanisms by which drugs induce attacks in porphyria-gene carriers are, however, even more complex and almost certainly multifactorial [167]. There are also interindividual differences between AIP-gene carriers, since some carriers may tolerate drugs that in others precipitate porphyric attacks and are listed as porphyrinogenic [137]. Cumulative exposure to various trigger factors might result in an AIP attack, even if the last trigger factor that is added is not strongly porphyrinogenic.

Symptoms and signs of porphyric attacks

In view of the protean manifestations, AIP has been called " the little imitator" (Waldenström), comparing it with the large "imitator" from the first part of the 20th century, syphilis. Unfortunately, there is no international consensus on a definition of an AIP attack. A combination of a) positive heredity, b) symptom constellation attributable to, or prominent in, AIP attacks (not chronic symptoms), c) intermittent course of the symptoms, when applicable, d) levels of porphyrin precursors above the reference limit and e) no other obvious cause of the symptoms should be regarded as an AIP attack.

Approximately 80-90% of AIP-gene carriers are reported to remain clinically asymptomatic throughout their lives [11]. According to other studies [12, 137], 40-50% of AIP-gene carriers have experienced symptoms of AIP. Females are more often affected than males [98, 137, 252, 257, 263, 273] and female sex hormones may play an important role in the manifestation of the disease [7, 15]. The female/male ratio of symptomatic cases is reported to be between 3:2 [15] and 4:1 [57] and 5:1 [75] to 9:1 [69].

Symptoms of AIP are rare before puberty, but they may occur [21, 124, 144]. In the last of these studies, children < 18 years of age were followed for 2.5 years and clinical evidence of AIP attacks was found in at 10% of the children.

Attacks of AIP may last from a few days to some weeks. In addition, the number of attacks varies considerably and, in some cases, a chronic porphyria syndrome develops.
The main clinical manifestations of AIP attacks in published studies are listed in Table 1.

Symptoms seen during acute attacks of porphyria presumably originate from effects on the nervous system [11, 96, 185, 190, 249]. Dysfunction of the autonomic nervous system may cause abdominal pain, constipation, vomiting, hypertension and tachycardia. Urine retention, fever and tremor may also occur. Motor neuropathy is the most common involvement of the peripheral nervous system and may involve the cranial nerves. Bulbar paralysis, respiratory paresis and death may occur. Dysfunction of the peripheral nervous system may also cause sensory symptoms with pain and/or paresthesia. Involvement of the central nervous system (encephalopathy)[263] may result in psychiatric disturbances, aphasia, apraxia, disturbed consciousness and epileptic seizures [80]. Low levels of serum sodium and magnesium are sometimes seen during acute attacks of AIP and may contribute to symptoms, such as signs of metabolic encephalopathy with weakness, lethargy, restlessness, confusion, delirium and seizures [11], as well as cardiac arrhythmias [218, 237].

Table 1. Clinical manifestations of acute attacks of porphyria in published studies.

Signs and symptoms	1 n=252	2 n=50	3 n=40/34*	4¤ n=88	5# n=51/22**	6¤ n=112/24**
Abdominal pain	85	94	95	95	96	97
Vomiting and nausea	59	88	43	80	84	79
Constipation	48	84	48	80	78	27
Hypertension	40	54	36	55	57	74
Tachycardia > 80/min	28	64	80	85	79	38
Mental symptoms	55	58	40	40	8	1***
Pain in the head and neck	n.a.	52	50	70	19	n.a.
Pareses/muscle weakness	42	68	60	50	8	46/10**
Epileptic seizures	10	16	20	20	2	5

1. Waldenström 1957 [257] 2. Goldberg 1959 [98] 3. Stein and Tschudy 1970 [238] 4. Mustajoki 1976 [188] 5. Mustajoki and Nordmann 1993 [189] 6. Hift and Meissner 2005 [122]
*40 cases with full information and 34 with partial information available. **Attacks/patients.***Only psychosis included. ¤ AIP and VP. #Cases treated with heme arginate. n.a. not applicable

Abdominal pain is the most common initial symptom and may mimic an acute surgical abdomen [98, 185, 238, 257]. Constipation, vomiting and diarrhoea often accompany the abdominal pain. In severe attacks of AIP, the urine invariably develops a red colour due to the high content of porphyrins and porphobilin, an auto-oxidised product of PBG.

Transient hypertension accompanies 30-50% of acute porphyric attacks [98]. This may be due to increased catecholamine secretion [230], or a dysregulation of the baroreceptor reflex [138] analogous to the sporadic hypertension of the Guillain-Barre syndrome [196]. Structural damage to the hypothalamus and brain stem nuclei has been described in patients who have died during AIP attacks [96]. Damage to these areas may cause changes in baroreceptor function and thereby more sustained hypertension [245].

Heilman and Muller [116] reported renal tubular damage in acute intermittent porphyria. It has been suggested that excess porphyrins or their precursors may be nephrotoxic [16, 65, 127, 137]. It has been suggested that the increased prevalence of hypertension in MAIP-gene carriers results from the presence of large amounts of porphyrin metabolites causing cytotoxic or vasospastic renal lesions, thus inducing an increase in blood pressure [150].

Hypertension and renal impairment have been the cause of considerable morbidity and mortality in the Chester kindred with its dual enzyme deficiency, PBGD and protoporfyrinogen oxidase deficiencies [54].

Anxiety, restlessness, paranoia, depression, confusion and hallucinations are not uncommon in AIP. Wetterberg [263] found a genuine AIP mental syndrome, probably originating from an organic brain syndrome with slight to moderate depression, transitional confusion, frequent

visual hallucinations and neurological signs. Depression and anxiety may become chronic and it may be difficult to distinguish between a primary psychiatric disorder and symptoms secondary to porphyria.

Hyponatremia is fairly common during acute attacks of porphyria and is sometimes part of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Damage to the supraoptic nuclei of the hypothalamus has been noted at autopsy [202]. Hyponatremia during acute attacks is not, however, always due to inappropriate antidiuretic hormone secretion. It has been suggested that salt depletion from gastrointestinal loss, combined with poor intake and excess renal sodium loss, may be more frequent causes of hyponatremia [237, 251]. Hypomagnesemia may also accompany acute porphyric attacks, as well as hypercalcemia due to inactivity [25, 252].

Epileptic seizures may occur in 10-20% of AIP-gene carriers [99, 238]. These numbers derive from highly selected clinic-based studies and include case reports of or brief comments on seizures [33, 34, 41, 64, 103, 120, 149, 154, 166, 194, 225, 242, 270]. The seizures may be generalised and due to systemic metabolic factors resulting from hyponatremia or other electrolyte disturbances leading to cerebral oedema or increased cortical excitability [19, 194, 195] or partial seizures due to focal ischemic changes caused by spasm in cerebral vessels [30, 39, 72, 117, 139, 147, 148, 204, 253].

Chronic symptoms/late effects

In 40% of patients who have had AIP attacks, hypertension may become sustained between attacks. Chronic renal failure occurs and hypertension may constitute a factor in the pathogenesis of such impairments [16, 54]. Some AIP-gene carriers display a chronic porphyria syndrome with peripheral and autonomic neuropathy, with or without a history of previous acute attacks [151, 190]. The symptoms are usually unspecific and composed of abdominal and muscle pain, weakness and fatigue and no specific treatment is available.

In AIP, structural and functional alternations in the liver are generally slight [98]. Increased bromsulphtalein retention and elevated aminotransferases have been reported during AIP attacks [197]. Hepatocellular carcinoma (HCC) is a common late accompaniment of the disease [6, 13, 29, 105, 112, 136, 160]. When hepatocellular carcinoma is established in AIP, the non-malignant liver tissue may be normal histologically and other cancer-predisposing factors such as liver cirrhosis are only present in 20-40% of the cases [6, 38].

The pathogenesis behind the high prevalence of HCC is not known. A reduced free heme pool could affect cytochrome P450, leading to an increase in reactive oxygen species and mutation rate. The long-term effect in hepatic tissue may lead to liver cell injury and the development of HCC. Furthermore, the auto-oxidation of ALA increases reactive oxygen radicals and causes the intrisic production of mutagenic substances [26].

Treatment

It is important to avoid factors that may precipitate an attack, such as stress, alcohol, certain drugs and chemicals, sex hormones including contraceptive pills, infections and low caloric intake. When symptoms of AIP occur, it is advisable to increase carbohydrate intake, which may reduce the excretion of ALA and PBG in urine [73, 84, 261]. The underlying mechanism of the "glucose effect" has recently been explained: the glucose down-regulates perioxisome-profilator-activated receptor γ co-activator 1α (PGC-1α), a protein, which directly induces the transcription of ALAS1 [111]. The clinical response to high carbohydrate intake varies considerably [260], and the use of glucose is limited to mild attacks, according to the guidelines for the treatment of an acute attack [9, 122].

Exogenous heme down-regulates ALAS in the liver (ALAS1) [132] and reduces the production of porphyrin precursors. On clinical grounds, hematin and its successor, heme arginate, are considered to be of great value in the treatment of acute AIP attacks [189, 260], although this efficacy has not been documented in controlled clinical trials [120]. The therapy should be started without delay [10, 189] in moderate and severe attacks. Potential side-effects of heme administration (in the form of either hematin or heme arginate) are mild coagulopathy [232] and thrombophlebitis [189], as well as anaphylactic shock in one case [62]. High doses of heme may lead to acute, reversible renal failure [61]. In recent years, vein problems and iron overload have been observed after repeated heme arginate treatments.

One option to restore hepatic PBGD activity to normal is liver transplantation. In 2004, Soonawalla *et al*. reported [236] a liver transplantation in a 19-year-old women who was severely stricken by acute AIP attacks. After the transplantation, the concentration of heme precursors in the urine returned to normal and 18 months later her quality of life was good.

Prognosis

AIP-gene carriers with previous episodes of porphyric symptoms run an increased risk of hypertension, renal dysfunction [16, 54] and hepatocellular carcinoma [6, 13, 29, 38, 105, 112, 136, 160]. After improved management, severe neuropathies and death during attacks are uncommon for AIP in Sweden and for AIP- and variegate porphyria-gene carriers in Finland [137] and South Africa [122].

A US report disclosed a three-fold increase in mortality rate among 136 patients with AIP as compared to the general population [129]. The increased mortality was related to the porphyric attack itself. Respiratory paralysis was the leading cause of death. Suicide rates were higher than in the general population. The survival rate increased after hematin therapy became available, but the difference before and after it became available did not reach statistical significance. No death was due to hepatocellular carcinoma; this is in contrast to Scandinavian studies. In reports from Argentina [69] and Chile [186], approximately 15% died during an acute porphyric attack, usually during the first attack and as a result of respiratory failure. In a recent prospective study of 12 Russian AIP-gene carriers, two of whom died during an acute attack, the clinical features and prognosis were discussed. Muscle weakness, impaired consciousness, hyponatremia, need for mechanical ventilation and bulbar paralysis were associated with a poor prognosis [205].

Pathogenesis of neurological dysfunction in AIP

The pathogenesis of neurological dysfunction in AIP is still not completely understood. It seems clear that a deficiency in the activity of PBGD is required, but such a deficiency *per se* is not sufficient to produce clinical manifestations. Erbslöh [79] first described axonal degeneration and patchy demyelisation of the femoral nerve. The patient had become paralysed after sulphonal medication. Since then, histological findings in the peripheral and autonomic nerves have included oedema, irregularity of myelin sheaths, thinned and irregular axons, axonal vacuolisation, degeneration and cellular infiltration [170, 218]. In the CNS, the vacuolisation of neurones, focal perivascular demyelisation and reactive glial proliferation have been described. Post-mortem pathological findings appear to bear little relation to the clinical symptoms [244]. In general, axonal degeneration and central chromatolysis are the most characteristic histopathological lesions in acute porphyria. Most investigations were conducted many years ago and the application of modern morphological histochemical and ultrastructural techniques has not been reported.

The main hypotheses concerning neurological involvement in AIP are as follows.

1. The accumulation of neurotoxic porphyrin precursors – ALA and/or PBG and/or derivatives of these
2. Deficiency of heme in neural tissue
3. Depletion of essential substrates or co-factors in the heme biosynthetic pathway, such as pyridoxal phosphate, zinc or glycine

The last of these theories has only limited supporting evidence and most studies have focused on the first two hypotheses.

1. <u>Accumulation of the neurotoxic precursors – ALA and/or PBG – and of porphyrins</u>

1.1 5-aminolevulinic acid

Intermediates of the heme synthetic pathway may accumulate in the brain due to insufficiency of the PBGD enzyme, or secondary to CNS uptake from tissues outside the blood brain barrier because of the increased peripheral concentration. Increased levels of ALA and PBG have been reported in the cerebrospinal fluid (CSF) during an acute attack [42, 100, 201, 244]. The administration of ALA to the animal, as well as the combined administration of ALA and PBG, leads to behavioural abnormalities, with increased locomotor activity and the induction of seizures [76, 173, 203]. It is, however, doubtful that the increased amounts of ALA and PBG in the CSF seen during acute attacks are sufficient to cause neurological dysfunction [100, 201].

Some investigators have concluded that ALA may cause neurological dysfunction due to a neuropharmacological action. *In vitro,* ALA is a partial agonist to presynaptic gamma-amino butyric acid (GABA) auto-receptors, reducing the presynaptic release of this inhibitory neurotransmitter [46]. This may contribute to seizures, but *in vivo* evidence of the effect of ALA on GABAergic systems is lacking. ALA exerts an effect on melatonin secretion from the rat pineal gland [210] and causes a marked decrease in daytime and night-time serum melatonin concentrations in symptomatic AIP-gene carriers [209]. 5-aminolevulinic acid also inhibits glutamate uptake and stimulates the release of this excitatory neurotransmitter in the rat [46, 175]. Aminolevulinic acid is an inhibitor of Na+, K+-ATPase activity [27]. In the gastrointestinal

tract, ALA causes a dose-dependent contractile response at low concentrations and complex interaction between ALA and GABA and glutamate receptors in the myenteric plexus has been suggested [60].

1.2 Porphobilinogen

Porphobilinogen probably plays no role in the pathogenesis of the neurological manifestations seen in porphyria. Patients with porphyria due to ALAD deficiency or tyrosinemia produce large amounts of ALA, while the levels of PBG are not elevated and therefore display the same neurological symptoms as the other acute porphyrias [177]. Furthermore, there is no evidence of any pharmacological activity by PBG in *in vitro* experiments. In a recent clinical study, PBG levels were reduced after the infusion of recombinante PBGD, but no clinical effects were found [17].

1.3 Neurotoxic products derived from ALA and PBG

5-aminolevulinic acid may undergo enolisation and auto-oxidation, reactions which, in the presence of heavy metals, may lead to the considerable generation of free oxygen radicals [182, 248]. Oxidative damage to isolated liver mitochondria takes place in the presence of ALA *in vitro* [118]. Injections of ALA in rats give rise to increased superoxide dismutase (SOD) activity, increased calcium uptake in cortical synaptosomes and signs of protein and lipid damage [71]. These *in vitro* studies have, however, been performed with ALA concentrations far above the concentrations occurring in the blood and CSF of patients during an acute porphyric attack. The hypothesis that transient mitochondrial damage induced by ALA causes some of the symptoms seen in porphyria therefore requires further investigation.

1.4 Porphyrins

Porphyrins may be toxic to nerve tissue [220]. However, the increased production and accumulation of porphyrins seen in other porphyrias with no increase in ALA are not associated with neurological dysfunction.

2. Deficiency of heme

2.1 In neuronal tissue and/or liver

A critical deficiency of heme could lead to reduced levels of key heme proteins, such as cytochromes and nitric oxide synthase (NOS), resulting in direct or indirect effects on the nervous system, see Figure 2, **A**. In the liver, a regulatory heme pool controls the activity of ALAS 1, the rate-limiting enzyme of the pathway. If the heme requirements of the cells are increased, ALAS is induced. It is not known whether brain ALAS is regulated by a similar mechanism. The precipitation of acute neurological symptoms from the CNS is presumably not caused by heme deficiency, as brain ALAS in rats is not induced by phenobarbital [67] and as heme administered intravenously to patients is not detected in the CNS [152]. The question of neural heme deficiency remains unsettled.

There is, however, evidence of a reduction in the function of heme proteins, such as hepatic tryptophan pyrrolase (TP) and hepatic cytochromes P 450 in AIP, see Figure 2, **B**. A lack of heme in liver cells may inhibit the breakdown of tryptophan as a result of reduced TP activity.

The blood level of tryptophan rises, increasing the supply of tryptophan to the brain where it functions as a substrate for tryptophan hydroxylase, thereby increasing serotonin (5-OH tryptamine, 5-HT) formation, see Figure 2, **C**. The increased excretion of the serotonin breakdown product, 5-hydroxy indolacetic acid (5-HIAA), in urine has been reported during acute attacks of porphyria [40, 207]. Puy *et al*. reported elevated blood levels of tryptophan and 5-HT and the increased urinary excretion of 5-HIAA in AIP-gene carriers and in rats [210]. These changes in tryptophan metabolism were reversed by heme arginate infusions [209, 210]. A change in serotonin metabolism is thus described in acute porphyria.

Increased amounts of serotonin in the brain may account for some of the symptoms observed in porphyria, such as nausea, abdominal pain, dysuria, psychomotor disturbances [24, 123, 233, 247] and depression and anxiety [268], see Figure 2, **H**. Serotonin receptors have a wide distribution and the activation of these receptors may result in the contraction or relaxation of intestinal smooth muscles, cardiac arrhythmias and hypertension [114], see Figure 2, **J**. Increased serotonergic activity may thus play a role in the clinical manifestations seen in porphyria, particularly when it comes to autonomic neuropathy.

Increased amounts of tryptophan (Fig 1, **G**) and its metabolites may impair hepatic gluconeogenesis, glycogen and glucose formation [179, 207, 234, 235, 254], see also the discussion on AIP and diabetes mellitus in Paper III. Tryptophan may in fact induce hypoglycaemia [2, 35, 63, 92, 106, 179, 269]. Glucose loading leads to higher levels of blood lactate and pyruvate in AIP-gene carriers in remission than in controls [119].

In the liver, the largest proportion of synthesised heme is used for microsomal cytochrome P 450, a group of enzymes which catalyses the oxidative metabolism of numerous exogenous and endogenous agents. Drug metabolism in subjects with AIP has been investigated by the clearance of antipyrine [8, 36]. The clearance of the drug is reduced in AIP- and variegate porphyria-gene carriers. Heme treatment can correct the oxidative metabolism of drugs in AIP [191]. There is, however, no evidence that a reduction in hepatic oxidative metabolism directly causes neurological symptoms in acute porphyria, but the possibility that impaired cytochrome P 450 activity in the brain or other nervous tissue may affect the P 450-mediated metabolism of neuroactive compounds and change neural transmission cannot be excluded.

2.2 Multifocal ischemia

Symptoms of acute porphyria, such as changes in gut motility, hypertension and tachycardia and alterations in pain perception, may relate to the impaired activity of either neuronal or vascular nitric oxide synthase (NOS), see Figure 2, **A** [130]. This cytochrome P 450 type heme protein [266] catalyses the five-electron oxidation of L-arginine to citrulline and nitric oxide (NO), see Figure 2, **A**. Nitric oxide is a short-lived free radical gas that is a neurotransmitter in the brain and peripheral nerves. It activates soluble guanylyl cyclase [181] and is involved in vasodilatation, neuronal signalling and host response to infection [45, 125, 163, 168], see Figure 2, **D**. The dysfunction of NO may be involved in various diseases such as multiple sclerosis [22, 228, 229, 272] and Parkinson´s disease [146, 180]. The proposed role of NO in these disorders is an induction of proinflammatory cytokines, or oxidative stress giving rise to neurodegeneration.

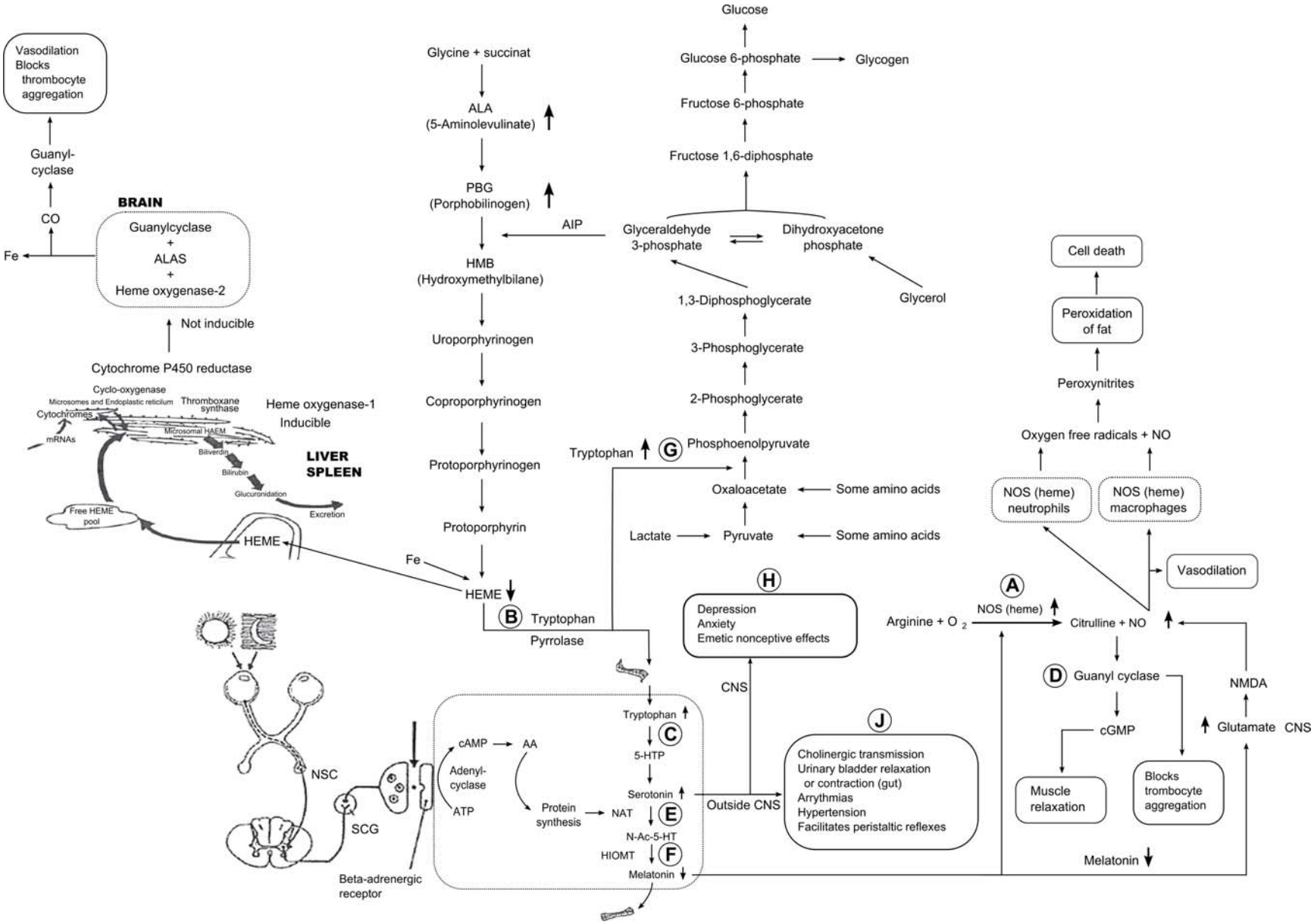

Figure 2. Schematic representation of metabolic interactions due to AIP.

Reduced activity of nitric oxide synthase may contribute to vasospasm and thus induce ischemic lesions in AIP [147]. Vascular cerebral dysfunction in AIP was first suggested in the 1940s and was partly based on autopsy findings [30, 72, 117]. Signs of vasospasm occur peripherally in both skin and retinal vessels during acute attacks of AIP and increases in blood pressure are well known. Reversible cortical lesions have been demonstrated on MRI during AIP attacks [1, 39, 139, 147, 253], similar to those of malignant hypertension [97].

However, the reversible cerebral lesions on MRI may relate to partial epileptic seizures [224]. Yet another possible cause may be the overly rapid correction of hyponatremia [241]. However, hyponatremia has only been described in one case of reversible cortical lesions. Sodium levels have not been specified in the other case reports [1, 39, 139, 147, 253].

Melatonin is synthesised in the pineal gland, from tryptophan, via serotonin, and acetylated by N-acetyltransferase (NAT, Figure 2, **E**) to N-acetyl-5-metho-oxytryptamine and converted to melatonin by the enzyme hydroxyindole-O-methyltransferase (HIOMT, Figure 2, **F**). The pineal hormone is linked to the light-dark cycle with low daytime and high night-time NAT activity. Despite increased levels of tryptophan in AIP-gene carriers, Puy *et al*. [209] demonstrated abnormally low levels of melatonin in plasma in a study of 12 symptomatic AIP-gene carriers, all women. The authors proposed that the biochemical mechanism could be a "GABA"-like effect of ALA that reduces NAT activity (Figure 2, **E**) and blocks the pineal response to beta adrenergic stimulation.

Melatonin inhibits NOS (Figure 2, A) [32, 206] and, as a result, low levels of melatonin may increase the level of NO and peroxynitrites in nervous tissue, leading to lipid peroxidation and neuronal death. Low levels of melatonin may also increase the levels of glutamate, which may also increase the levels of NO [32, 206]. Low levels of melatonin may thus counteract the vasospasm due to low levels of NO. The essential role of NO in AIP, if any, is not known.

Summary of pathogenesis

To summarise; the most likely pathogenesis of symptoms in AIP relates to heme deficiency in nerve cells, linking the biochemical, clinical and neuropathological findings in the disorder. However, ALA may also act as a pharmacological or neurotoxic agent in this disease, perhaps augmenting the effects of heme deficiency.

Aims of the studies

I. To demonstrate the prevalence of various forms of epileptic seizure in an unselected population of AIP-gene carriers and to evaluate the relationship of the seizures to AIP and to other factors

II. To investigate melatonin production in AIP-gene carriers with and without known epileptic seizures and to study whether melatonin may have an anti-convulsive or a pro-convulsive effect in AIP

III. To investigate the effects of diabetes mellitus on the clinical expression of AIP

IV. To investigate whether AIP-gene carriers with and without previous porphyric attacks, examined in remission, display white-matter lesions on brain magnetic resonance imaging and/or abnormalities in plasma and cerebrospinal fluid, including oligoclonal bands, and to investigate a possible relatioship with multiple sclerosis

V. To identify the underlying genetic defect in each AIP-affected family, to describe the clinical symptoms and the severity of the symptoms of AIP attacks and precipitating factors, to describe concomitant diseases, relevant laboratory values and sick leave and disability pension due to AIP and thereby update the clinical course of the disease in order to offer the carriers improved treatment and quality of life

Patients and methods

Diagnostic criteria

The diagnosis of AIP was based on the increased excretion of ALA and PBG in urine, the reduced activity of erythrocyte PBGD, mutations in the PBGD gene, or a combination of these factors [249].

Diagnosis of AIP

In **Papers I-III and V,** the diagnosis of AIP was established at the Porphyria Centre Sweden at Huddinge University Hospital, Stockholm, Sweden. For **Paper IV,** the biochemical diagnosis of AIP was established at Trondheim University Hospital, Norway.

Diagnosis of diabetes mellitus

Diabetes mellitus was defined according to the WHO criteria [267]. In **Paper III,** screening for diabetes mellitus in AIP-gene carriers previously not known to be diabetic was performed by determining the fasting blood glucose concentration, with a cut-off level corresponding to 6.2 mmol/l.

Study population and selection of patients

Paper I. A questionnaire was sent to all known Swedish AIP-gene carriers who (1996) were in the records of the Porphyria Centre Sweden in Stockholm at that time (n=294) and 268 (91%) responded to the questionnaire. To avoid transmission to deceased persons, the Official Swedish Population Record was consulted. The subjects were asked whether they had had previous attacks of porphyria, epileptic seizures, or a diagnosis of diabetes mellitus.

Paper II. A questionnaire was sent to the ten AIP-gene carriers who had experienced epileptic seizures and were included in the study presented in **Paper I** and to three relatives of each carrier. The relatives were AIP-gene carriers but had not experienced epileptic seizures. The subjects were invited to submit morning samples of urine for the determination of melatonin excretion during two consecutive nights.

Paper III. The population area consists of the two most northerly counties of Sweden, i.e. Norrbotten and Västerbotten (550 000 inhabitants). In total, 433 AIP-gene carriers had been diagnosed at the end of a case-finding study, comprising most AIP-gene carriers in the area. Of these, 346 gene carriers were aged > 18 years and 328 (95%) agreed to participate. The evidence of AIP symptoms after the diagnosis of diabetes was based on individual anamnesis and medical records.

Paper IV. The population consists of all known AIP-gene carriers (n=60) in the county of Nordland (240,000 inhabitants) in northern Norway (1999). Of these, eight gene carriers with manifest AIP and eight carriers with latent AIP were selected.

Paper V. The population area consists of the four northernmost counties in Sweden with almost one million inhabitants. At that time, 1999, a case-finding study was completed and a total of 469 AIP-gene carriers had been diagnosed, comprising almost all the AIP-gene carriers in the area. Of these, 386 gene carriers were aged > 18 years and 356 agreed to participate (92%) in the study. In a follow-up study in 2001, 282 (79%) of the 356 gene carriers responded to another questionnaire.

Formulation and validation of the questionnaire

In **Papers I and II**, AIP-gene carriers were asked questions about whether they had had attacks of AIP and whether they had had epileptic seizures. The carriers who had suffered epileptic seizures received another questionnaire with more detailed questions regarding their seizures. The medical records of all gene carriers were scrutinised for the documentation of epileptic seizures and related information. Information from the questionnaire was double-checked against the medical records (searching for information on AIP attacks and seizures) of the 112 patients from northern Sweden. The validation did not reveal any false-negative or false-positive information about seizures. We were able to contact 17 of the 26 non-responders (i.e. 65%) by telephone or check their patient files. None had had seizures.

Sample collection and analysis of melatonin

The subjects reported in **Paper II** were supplied with graduated plastic beakers to measure urine volume and plastic vials for storage. The collection procedure included emptying the bladder at bedtime (10-11 pm), recording the exact time of voiding and then discarding the urine. The measurement beaker was then placed on the toilet cover and all the urine during the night, including the first morning urination (at about 7 am), was then collected in the graduated plastic beaker. The time of collection, the total urine volume and time of any nocturnal voiding was recorded. Part of the total urine volume was poured into the plastic vial, which was immediately transported to the laboratory in Stockholm by post. The vial arrived at the laboratory within 24 hours from delivery and was immediately frozen and stored at -20°C until assay. Urinary melatonin is stable under these conditions of transfer and storage [265] and urinary melatonin concentrations in samples collected in this manner correlate strongly with the 2 am peak value of serum melatonin ($n=64; r=0.8; p<0.001$) [5]. Since melatonin production occurs mainly at night and urinary melatonin concentration is in equilibrium with blood concentration [5], overnight urinary melatonin excretion may provide an integrated estimate of melatonin secretion.

Magnetic resonance imaging (MRI)

The AIP-gene carriers reported in **Paper IV** were investigated in a 1.5 Tessla Philips Gyroscan Intera ® with sagittal T1W, transverse T2W and sagittal and coronal T2W FLAIR sequences without intravenous contrast. These subjects also took part in a personal interview and a complete medical and neurological examination.

Investigation of plasma and cerebrospinal fluid (CSF)

In **Paper IV,** we set out to investigate whether AIP-gene carriers have biochemical abnormalities in plasma and cerebrospinal fluid, including oligoclonal bands.

Venous blood samples were drawn, collected on ice and centrifuged after adding heparin and were immediately analysed using standard biochemical analyses. All the carriers underwent a diagnostic lumbar puncture at the level of L3/L4 or L4/L5. The liquor was examined for cell count and protein, in addition to agarose gel electrophoresis with isoelectric focusing.

Examination and sample collection, Paper V

The standard biochemical laboratory analyses were performed at the accredited departments of clinical chemistry at the local hospitals in the region. Urinary delta-aminolevulinic acid (U-ALA) and porphobilinogen (U-PBG) were analysed at the University Hospital of Umeå. The DNA tests for mutation analyses in the PBGD gene were performed at the accredited laboratory at the Swedish Porphyria Centre, Stockholm, Sweden.

Summary of Papers I-V

In this part, quotation from own papers occur.

Paper I: Epidemiology and clinical characteristics of seizures in patients with acute intermittent porphyria

Aims

The primary aim of the study was to assess the prevalence of epileptic seizures in an unselected population of Swedish AIP-gene carriers. Additional aims were to evaluate the relationship of seizures to AIP or other potential trigger factors and to classify the types of seizure that occur.

Methods

See "**Patients and methods".**

Results

In total, 248 AIP-gene carriers, 141 women (mean age 49, range 4-83 years) and 127 men (mean age 45, range 3-83 years), responded to the questionnaire (91.2%). Eighty-two women (58%) (mean age 54, range 29-80 years) reported episodes of porphyric attacks, while 59 women (mean age 42, range 4-83 years) did not. Thirty-five men (28 %) (mean age 48, range 17-83 years) had had episodes of porphyric symptoms, while 92 men (mean age 44 years, range 3-83 years) had had no such symptoms.

Ten AIP-gene carriers reported epileptic seizures at some time in their life, corresponding to a prevalence of 3.7%, see Table 2 and Figure 3. Eight were women (mean age 54, range 30-81 years) and two were men. Six women had had previous episodes of porphyric symptoms (mean age 49, range 30-63 years), while the two men (9 and 29 years old respectively) had no history of porphyric symptoms. Six of the ten AIP-gene carriers have the W198X mutation in exon 10 and one has a mutation in intron 9 affecting splicing. In the remaining three cases, the mutation was not known at the time of the study.

Six of the individuals had experienced tonic-clonic seizures, while four had had partial seizures which became secondarily generalised.

Hyponatremia in serum was observed at the time of seizures in three individuals (mean 110mM/L, range 103-120 mM/L). Serum sodium concentrations were normal in five, while they were not reported in two of the AIP-gene carriers with seizures.

In six subjects, including the three with hyponatremia, the seizures took place during an acute attack of AIP. Carriers with AIP-associated seizures had a low seizure frequency and each subject had experienced between one and five seizures.

In four of the six carriers with AIP-associated seizures, ALA excretion in urine was investigated and found to be increased at the time of the seizures. A metabolic disturbance in the form of increased ALA in urine and/or disturbed serum electrolytes was thus noted in five

of the six carriers with AIP-associated seizures. The sixth carrier had normal serum electrolytes and no analysis of ALA in urine was performed at the time of the seizures.

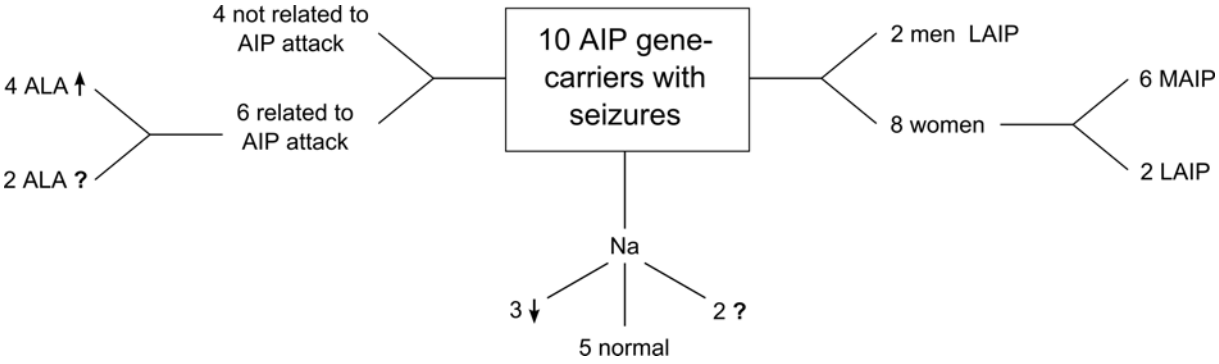

Figure 3. Schematic figure of the AIP-gene carriers with epileptic seizures.

Conclusions

The prevalence of seizures in AIP-gene carriers is 3.7% and the majority (2.2%) take place during an attack of AIP. A prevalence of 3.7% with regard to seizures is not very different from what is expected in the general population. However, the prevalence of 5.1% of seizures in carriers with MAIP is higher than in the general population. However, if hyponatremia alone was considered to precipitate seizures, only three had seizures attributed to AIP *per se* (2.6%). Carriers with AIP-associated seizures had a low seizure frequency and each had experienced one to five seizures.

Seizures occurred in 5.6% of the female and 1.6% of the male AIP-gene carriers. In carriers with a history of porphyric symptoms, 9.6% of the women and none of the men had experienced seizures. As a result, seizures are more common among women. Due to the small sample size, a general conclusion regarding age or type of seizure should be considered with caution. Epileptic seizures in AIP-gene carriers are less common than has previously been reported. When they occur, they are most frequently related to an AIP attack.

Table 2. Summary of statistics from ten AIP-gene carriers with epileptic seizures.

Case	1	2	3	4	5
Sex	M	M	F	F	F
Age at study (yr)	9	29	60	81	50
Age at AIP diagnosis (yr)	1	27	?	79	27
Type of AIP mutation	W198X	W198X	W198X	W198X	?
Acute AIP symptoms					Abd. pain
No. of AIP attacks					3
AIP trigger factors					Drugs Fasting
Age at first seizure year	11 months	3	58	52	41
No. of seizures	>25	5	1	7	2
Seizure-provoking factors	Infection	?, Metanolintox	Head trauma	?	Fasting
Electrolytes at time of seizure	?	Normal	Normal	?	Normal
ALA excretion at seizure	?	?	?	?	Increased
Seizure related to AIP	No	No	No	No	Yes
Seizure type	Part sec gen	Tonic-clonic	Part sec gen	Part sec gen	Part sec gen
AED treatment	DZP, PHT, VPA	PB, DZP	None	PHT	DZP

Case	6	7	8	9	10
Sex	F	F	F	F	F
Age at study (yr)	63	51	48	50	30
Age at AIP diagnosis (yr)	24	22	24	45	21
Type of AIP mutation	Intron 1	?	?	W198X	W198 X
Acute AIP symptoms	Abd. pain, HT/TC	Abd. pain, back pain	Abd. pain, HT/TC, psychosis	Abd. pain	Abd. pain, co, pa HT/TC, psychosis,
No. of AIP attacks	1	1	>15	>8	>100
AIP trigger factors	Drugs, operation	Drugs	Mens.	Mens, fasting, infection	Mens, infection
Age at first seizure year	24	25	24	49	26
No of seizures	3	1	1	1	5
Seizure-provoking factors	Drugs, operation	Drugs	Delivery, pain	?	Infection
Electrolytes at time of seizure	Na 120	Normal	Normal	Na 103	Na 107
ALA excretion at seizure	?	?	Increased	Increased	Increased
Seizure related to AIP	Yes	Yes	Yes	Yes	Yes
Seizure type	Tonic-clonic	Tonic-clonic	Tonic-clonic	Tonic-clonic	Tonic-clonic
AED treatment	?	None	None	Chlormeth	Chlormeth

Legend: AIP. Acute intermittent porphyria: ALA. 5-aminolevulinate: ? unknown: HT. hypertension : TC. tachycardia: pa. Paresis. part sec gen. partial secondarily generalised: co. constipation: AED. anti-epileptic drugs: Na. sodium in mM: PHT. phenytoin: PB phenobarbital: VPA: valproate : CZP. clonazepam CBZ. carbamazepine: Chlormeth. chlormethiazole. DZP: diazepam: M men: F female: Abd abdominal pain: Mens menstruation

Paper II: Melatonin and epileptic seizures in patients with AIP

Aim

To assess melatonin excretion in AIP-gene carriers with and without epileptic seizures and to address the question of whether melatonin may have an anti-convulsive or pro-convulsive effect in AIP.

Subjects and methods

Ten AIP-gene carriers who had experienced epileptic seizures and three gender-matched AIP-gene-carrying relatives of each carrier, who had not experienced epileptic seizures, were invited to send samples for measurements of melatonin concentration in urine during two consecutive nights.

Results

Eight AIP-gene carriers who had experienced epileptic seizures and 14 of their relatives without a history of seizures sent urine samples for evaluations of melatonin concentration.

Of the AIP-gene carriers with epileptic seizures, six were women (mean age 59, range 48-81 years). Four of them had a history of previous episodes of porphyric symptoms (mean age 53, range 48-63 years) and two had no history of porphyric symptoms (mean age 71, range 60-81 years). Two were men, nine and 29 years of age, both without a history of porphyric symptoms, see cases 1 to 8 in Table 2.

Of the 14 relatives with AIP without a history of epileptic seizures, 12 were women (mean age 63, range 41-86 years), nine had a history of previous episodes of porphyric symptoms (mean age 63, range 41-86 years) and three had no history of porphyric symptoms (mean age 47, range 6-81 years). Two of these relatives were men, 11 and 32 years of age, both without a history of porphyric symptoms.

The mean urine concentration of melatonin in the eight AIP-gene carriers who had experienced previous epileptic seizures was 128.8 nmol/L (SD 36.8, range 70-180) for the first night and 133.8 nmol/L (SD 55.8, range 50-210) for the second. The corresponding values for the fourteen controls were 220.0 nmol/L (SD 97.3, range 70-430) and 247.1 nmol/L (SD 140.4, range 50-150) respectively.

The AIP-gene carriers with epileptic seizures had a significantly lower concentration of melatonin in urine as compared to the AIP-gene carriers who had never had an epileptic seizure (night one $p< 0.014$, night two $p<0.043$; Wilcoxon's rank sum test).

Conclusions

The lower concentration of melatonin in AIP-gene carriers who had had epileptic seizures as compared to relatives without epileptic seizures may suggest a possible anti-convulsive effect by the endogenously produced melatonin.

Paper III: Effects of diabetes mellitus on patients with acute intermittent porphyria

Aim

To evaluate the effect of diabetes mellitus on the clinical activity of porphyria in AIP-gene carriers

Subjects and methods

A population-based study of all carriers with DNA-verified AIP aged > 18 years (n=346) living in the two most northerly counties in Sweden (Norrbotten and Västerbotten with 550,000 inhabitants). The frequency of attacks of AIP before and after establishing a diagnosis of DM was based on personal interviews and patient files. Screening for diabetes mellitus in AIP-gene carriers was performed by determining the fasting blood glucose concentration.

Results

Eighteen of the 346 AIP-gene carriers were not willing to participate and 328 gene carriers therefore participated (95%). According to their medical records, none of the excluded carriers had DM.

Sixteen AIP-gene carriers had DM (4.9%). Eight (women: men 5:3) had had previous symptoms of porphyria (mean age 64.6, range 50-72 years) and eight subjects (women: men 1:7) had no history of porphyric attacks (mean age 70.1, range 63-92 years). After the diagnosis of DM was established, no AIP-gene carrier in either group suffered from an acute attack of AIP.

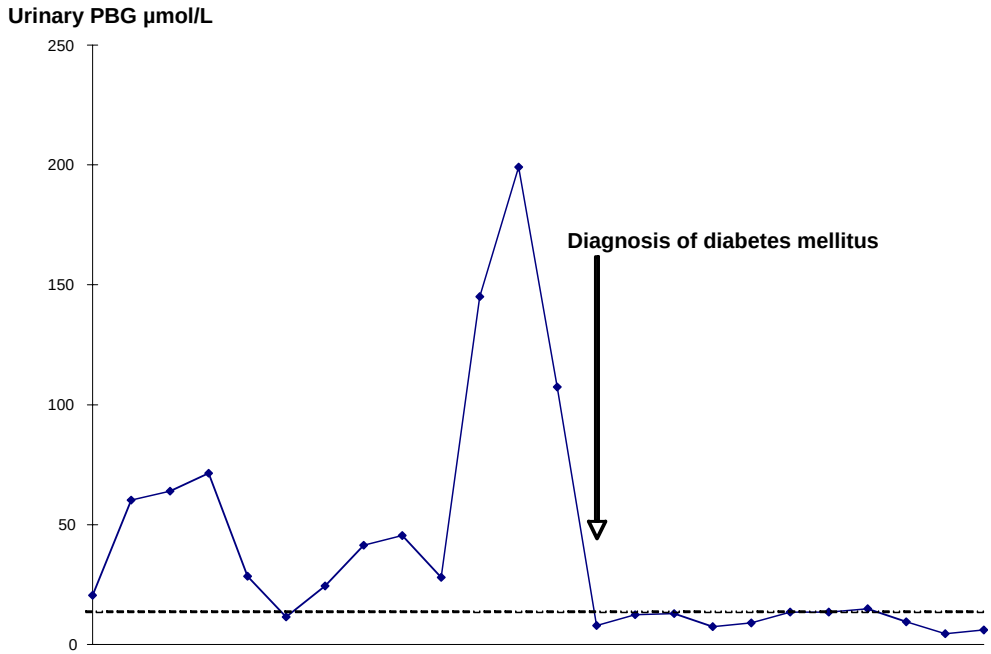

Figure 4. Urinary porphobilinogen levels of a 52-year-old woman with severe AIP during a six-month period before she developed type 2 diabetes and during the first four months after she became diabetic. ------- Upper reference level for urinary prophobilinogen (11µmol L^{-1}).

Conclusion

Sixteen AIP-gene carriers (4.9%) had DM. No acute attack of porphyria occurred after the diagnosis of DM was established. It is possible that the diabetic metabolism prevent attacks of AIP, even if the potential mechanism is unknown. It could be argued that the mean age of the carriers with DM in this study is somewhat high and the activity of AIP decreases with age. This might be true for women, but it should not be expected in men with MAIP.

Paper IV: Brain Magnetic Resonance Imaging, white-matter lesions and cerebrospinal fluid findings in patients with acute intermittent porphyria

Aim

Case reports include similarities such as limb weakness between multiple sclerosis (MS) and AIP. We explored whether AIP-gene carriers examined outside attacks display white-matter lesions on brain MR and/or abnormalities in plasma or cerebrospinal fluid (CSF) including oligoclonal IgG bands.

Subjects and methods

Eight AIP-gene carriers with previous attacks of porphyria with a mean age of 42.9 (range 30-60 years) and eight carriers without previous attacks with a mean age of 42.8 (range 33-62 years) from the county of Nordland, Norway, were investigated.

No carrier had a history of MS, vasculitis or migraine. All carriers had a normal neurological examination at the time of investigation.

Magnetic resonance imaging (MR) was performed with sagittal T1W, transverse T2W and sagittal and coronal T2W FLAIR sequences in a 1.5 T magnet (Philips Gyroscan Intera®). No intravenous contrast was given.

Venous blood samples were drawn, collected on ice and centrifuged after adding sodium heparin and were immediately analysed. Extra plasma was frozen at -20°C for future examination.

All the subjects underwent a diagnostic lumbar puncture. The liquor was examined for cell counts and protein, in addition to agarose gel electrophoresis with isoelectric focusing. An additional 5 ml of CSF was frozen and stored at -70°C in dark conditions for future examination.

Results

One 58-year-old male carrier with previous attacks of porphyria had multiple white-matter, high-signal lesions on T2W sequences, see Figure 5. In one male carrier, 35 years of age, with previous attacks of porphyria, a few (<10), scattered, rounded, white-matter, high-signal lesions were demonstrated on T2W sequences. The same was found in two other carriers, one male and one female without previous attacks of porphyria, both 60 years of age. The carrier

with multiple white-matter lesions and the woman with a few white-matter signals were being treated for hypertension. No subjects had oligoclonal bands at agarose gel electrophoresis in CSF or serum. One carrier had an increased level of protein in liquor (0.63 g/L, reference level <0.50 g/L). Seven subjects (44%), four without and three with previous attacks of porphyria, had increased levels of HbA1C (>6.0%). None had an established diagnosis of diabetes mellitus.

Conclusion

No AIP-gene carriers had oligoclonal immunoglobulin bands in liquor or plasma. Only one carrier had increased protein levels in liquor; this is in contrast to a study of AIP-gene carriers during acute attacks. One of four AIP-gene carriers displayed high-signal, white-matter lesions on brain MR when examined in remission. The lesions are unspecific but may possible relate to their porphyria. A diagnosis of AIP should be considered in patients with suspected MS or Guillan-Barre.

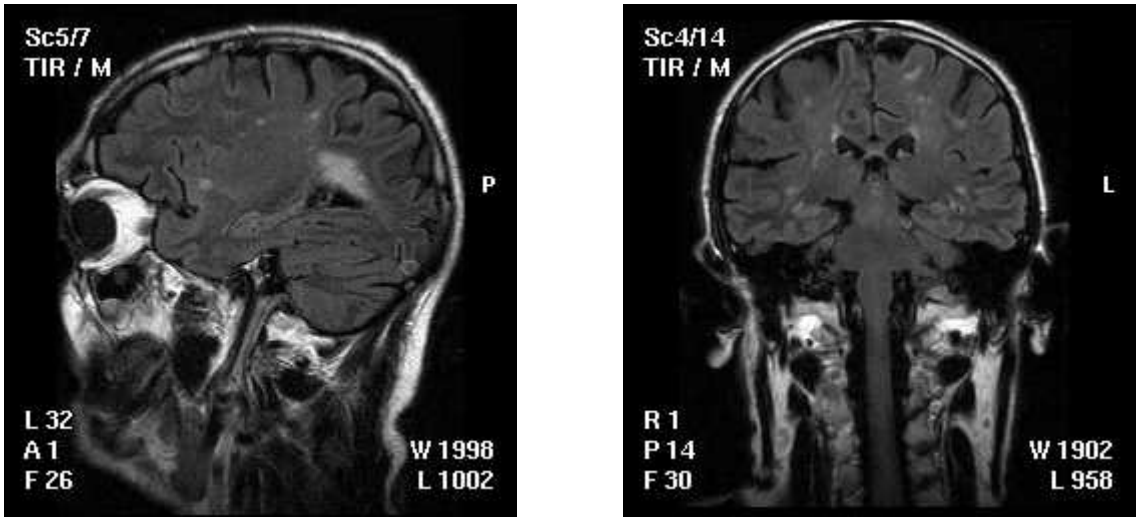

Figure 5. Sagittal and coronal FLAIR MR-images from a 58-year-old male with AIP (patient 1). The patient was examined outside attacks but has had multiple earlier AIP attacks and has been treated for hypertension for more than sixteen years. The MR images show multiple, symmetrical, white-matter, high-signal lesions consistent with demyelination.

Paper V: Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study

Aim

To identify the underlying genetic defect in each AIP-affected family and to describe the clinical symptoms and severity of AIP attacks, as well as trigger factors for AIP attacks, relevant laboratory values, observed concomitant diseases and sick leave and disability pension due to AIP.

Subjects and methods

A retrospective population-based study was performed in 1995-1999, covering the four northernmost counties of Sweden. All known AIP-gene carriers were invited to undergo a standardised investigation including medical history (verified by medical records), physical examination and laboratory tests. Questionnaires with open and closed questions were used. The main topics focused on AIP symptoms, age at first porphyric attack, duration and frequency of attacks, hospital admission due to attacks, precipitating factors, sick leave and disability pension, smoking and alcohol habits and laboratory tests relevant to the AIP disease. All the investigations were performed when the disease was in remission. Definition of an AIP attack: in this study, a combination of a) positive heredity, b) symptom constellation attributable to, or prominent in, AIP attacks (not chronic symptoms), c) intermittent course of the symptoms, when applicable, d) levels of porphyrin precursors above the reference limit and e) no obvious cause of the symptoms was regarded as an AIP attack.

Results

Three hundred and fifty-six AIP-gene carriers > 18 years of age (male: female 176: 180) participated in the study (92%); 149 (42%) had manifest AIP (65% were women) and 207 carriers had latent AIP, see Figure 6. The carriers with manifest AIP were significantly older and had a higher body mass index than carriers with latent AIP. Eight mutations in the PBGD gene were found, primarily W198X (88.9%) and R167W (5.8%). Women were more severely stricken by AIP attacks in terms of the number and duration of attacks, hospital admission and early onset. Men reported most attacks at > 40 years of age. The most common symptoms reported during attacks are shown in Figure 7: severe abdominal pain (86%), fatigue (42%), constipation (41%), vomiting (36%) and muscle pain (30%). Eighty-seven per cent reported at least one or two symptoms in addition to abdominal pain. Chronic AIP symptoms were reported by 18% of the gene carriers with MAIP. The most commonly reported precipitating factors are shown in Figure 8: menstruation (31%), psychological stress (30%), drugs (20%) and low caloric intake (19%). Smoking was associated with frequent AIP attacks. Half the gene carriers who were on medication used drugs that were not considered safe according to the Swedish recommendations in 1999, mainly diuretics, calcium antagonists and ACE inhibitors. Elevated levels of ASAT, bile acids, U-ALA and U-PBG and reduced levels of creatinine clearance were associated with MAIP after adjustment for age and sex, as were hypertension and pain in the legs.

Conclusion

Despite remarkable progress in the understanding of the genetic and pathophysiological factors behind the disease, this study, which was based on one of the largest populations of AIP-gene carriers in the world, reveals that 42 % of the investigated carriers had manifest AIP and that AIP is not a harmless disease. Early counselling is therefore essential to avoid the clinical and social burden of the PBGD mutation.

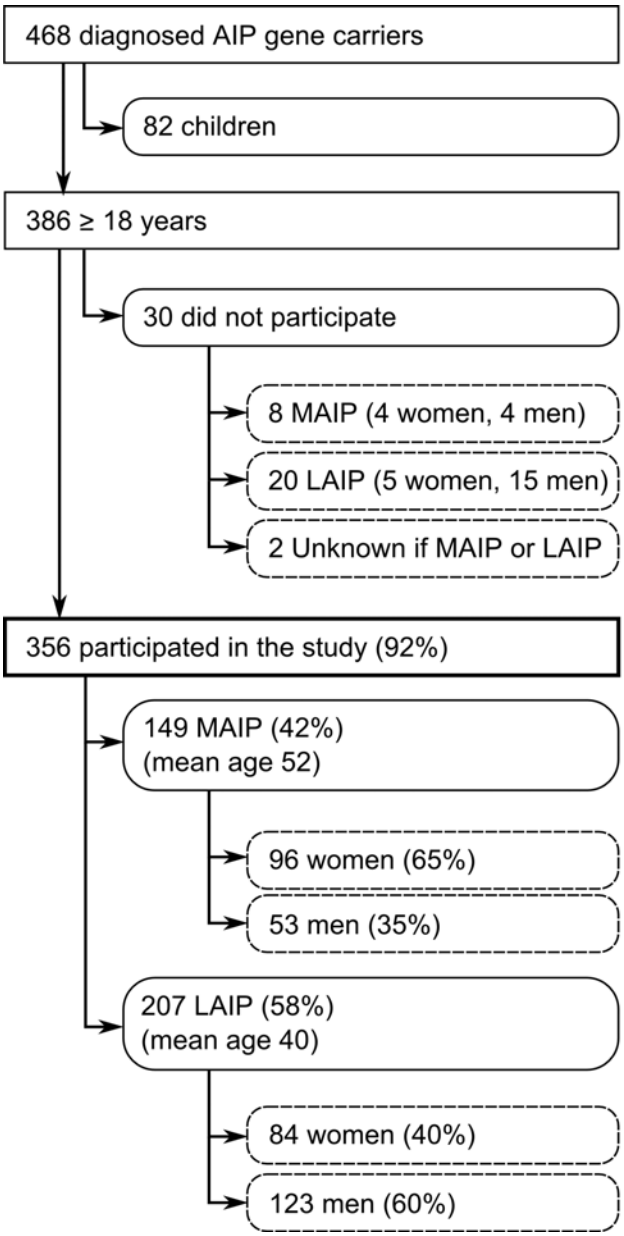

Figure 6. A population-based investigation to describe clinical aspects of acute intermittent porphyria.

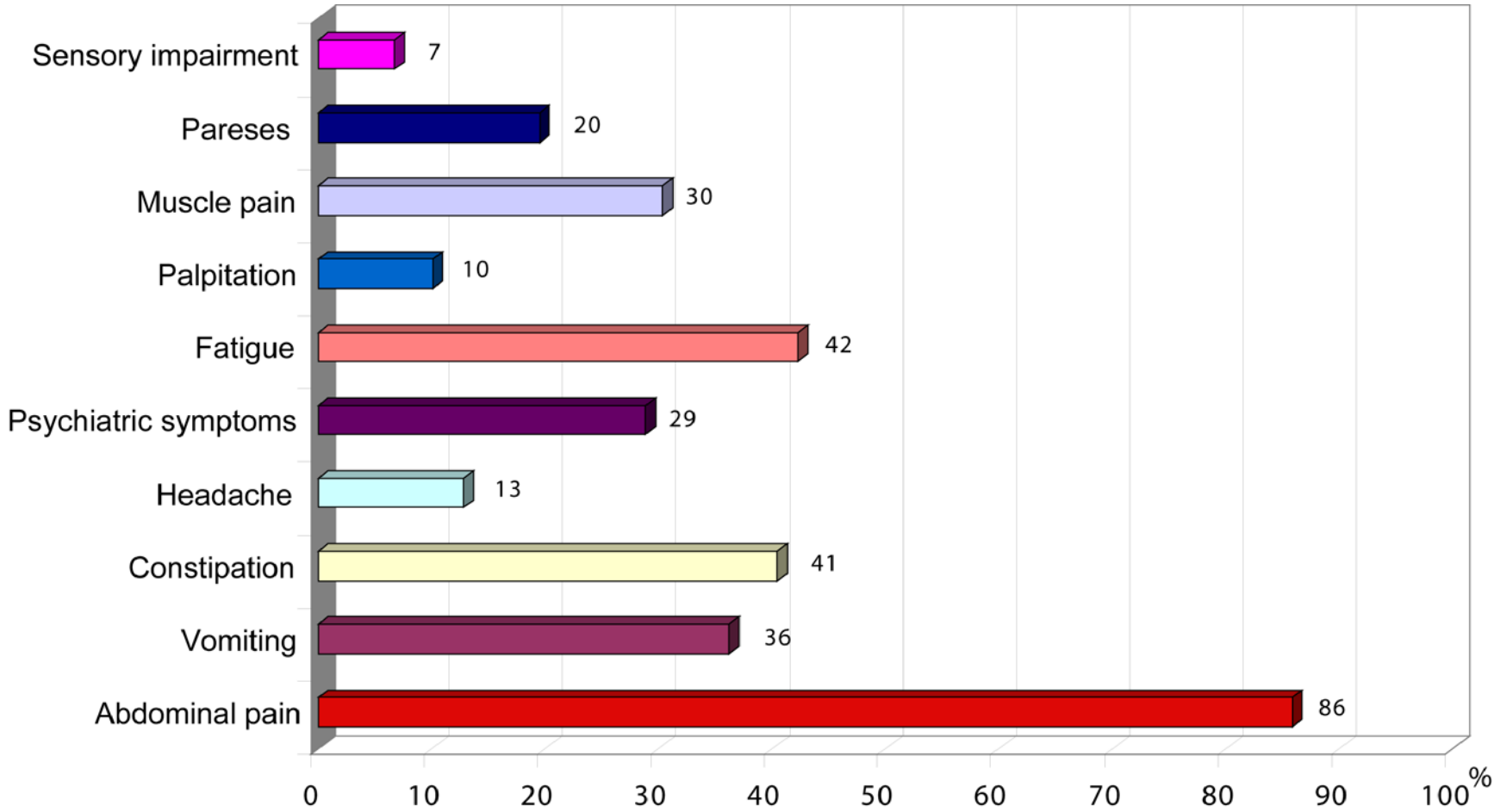

Figure 7. Frequency of symptoms (%) during acute attacks reported by 149 gene carriers with manifest AIP.

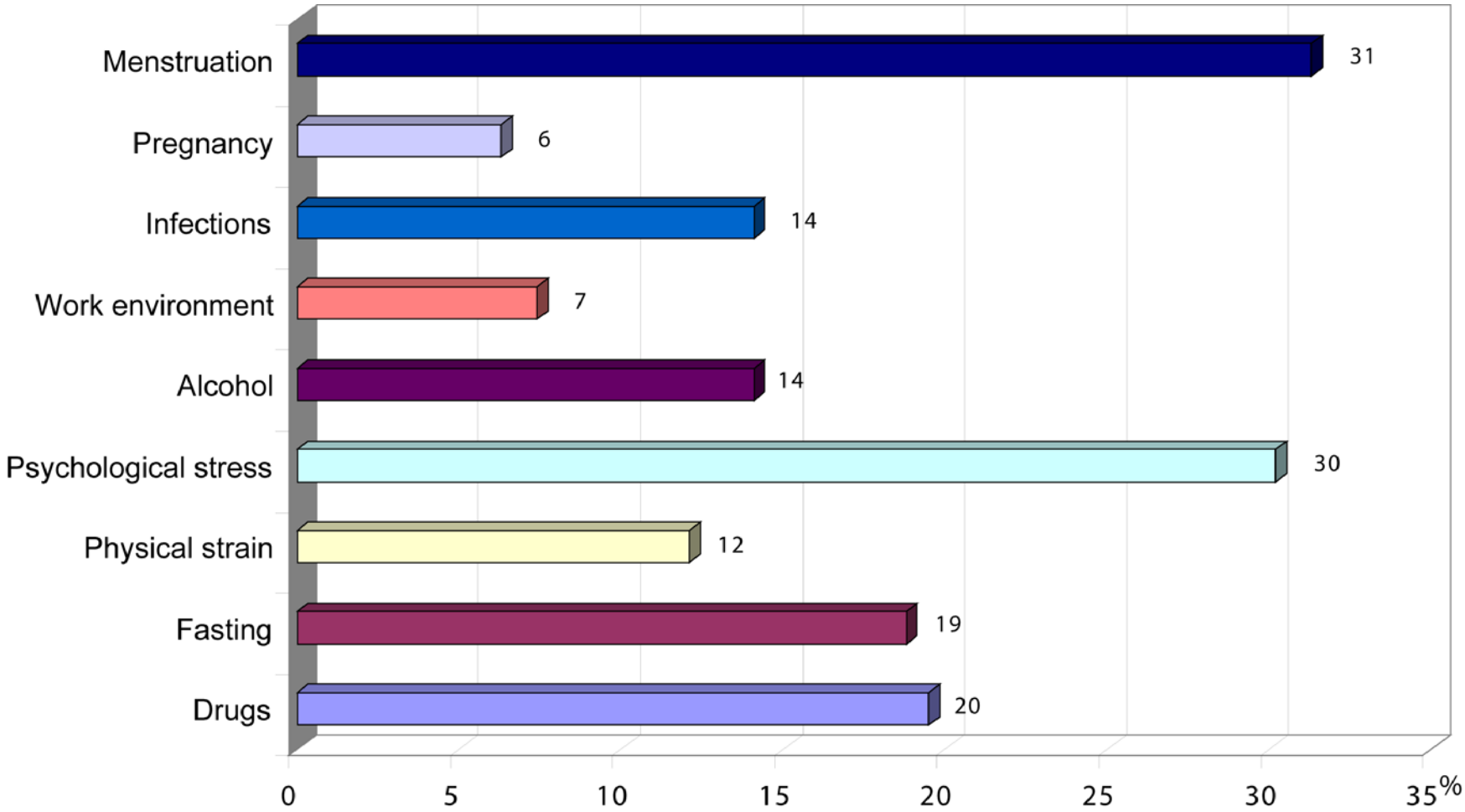

Figure 8. Frequency of precipitating factors (%) to AIP attacks reported 149 gene carriers with manifest AIP.

Discussion

AIP and epileptic seizures, Papers I and II

The word epilepsy is derived from the same Greek root as the verb *to seize*. This may refer to the ancient belief that an individual was *seized* by gods or demons during the attack. The oldest work on file on epilepsy is "On the Sacred Disease", a monograph from the medical teachings of Hippocrates. Hippocrates recognised two forms of epilepsy: convulsive and apoplectic. In the Middle Ages, epilepsy was referred to as the "falling sickness". The condition was associated with possession by the devil. During the Renaissance, the seizures were thought to be a manifestation of physical illness, primarily originating in the brain. Modern concepts of epilepsy have their origin in the writings of Jackson [169].

The discovery of the electroencephalographic (EEG) technique [44] established a neurophysiological basis for epilepsy. It became an important tool to distinguish epilepsy from other seizure conditions and to distinguish different seizure types [95]. Penfield and Jasper [199] first developed the modern concept that all epileptic seizures are accompanied by abnormal electric discharges in the brain.

Epilepsy is thus defined as a condition of abnormal neuronal firing [93], but the exact pathophysiological mechanisms are not known. It is generally thought that a balance between excitatory and inhibitory impulses normally maintains the normal neurophysiological activity in the brain and that impairment of GABAergic or an increase in glutamatergic function may lead to the development of seizures [176].

Epileptic seizures are recognized in a large number of clinical phenomena, some of which e.g. myoclonic and atonic seizures may reflect neuronal mechanisms different from the pathophysiological processes traditionally considered to be "epileptic". Epilepsy *su generis* ("by itself") conforms to the original Greek meaning of "idiopatic". Symptomatic epilepsies are those in which seizures are the consequence of an identable lesion or other specific etiology. If the epilepsy is presumable symptomatic but of unknown etiology they are termed cryptogenic [78].

During seizures, the levels of excitatory amino acids like aspartate, glutamate, glycine and serine increase in extracellular fluid [51, 176, 222]. Glycine may potentiate the effect of glutamate. Serine is not regarded as a neuroactive amino acid, but it is inter-convertible with glycine in the CNS. To the best of our knowledge, no study has investigated the levels of amino acids in the CSF during acute attacks of porphyria.

AIP

Between 10 and 20% of AIP-gene carriers suffer from epileptic seizures, according to studies by Waldenström in 1957 [257], Goldberg and Rimington in 1962 [99], Stein and Tschudy in 1970 [238] and Mustajoki in 1976 [188]. Previous reports of seizures in AIP are based on highly selective, clinically based studies and include case reports or brief comments [33, 34, 41, 64, 103, 120, 149, 154, 166, 194, 225, 243, 270].

The pathophysiological basis of the seizures in AIP-gene carriers is elusive. Hyponatremia and/or hypomagnesemia due to the gastrointestinal loss of electrolytes [166], overhydration or inappropriate ADH secretion [87] may contribute. We found low plasma levels of sodium and

magnesium at the time of seizures in three carriers during an AIP attack and normal levels of sodium in three carriers during an AIP attack, as well as in two carriers with LAIP. In addition to our findings, there are only a few case reports of low levels of sodium and magnesium during seizures [33, 34, 41, 64, 103, 120, 149, 154, 166, 194, 225, 243, 270].

The potential metabolic background of epileptic seizures in AIP-gene carriers is considered in more detail in the following text.

ALA

Serum ALA increases during an acute attack of AIP [183]. The intracerebroventricular administration of ALA in rodents induces convulsions and death [203, 231], but acute systemic injections of ALA cause only mild locomotor effects [59, 173], presumably due to low blood brain barrier permeability to this compound [174]. Cerebral tissue may accumulate ALA [133]. ALA may be produced within the brain [67, 134] and cerebral ALA levels may thus be higher than those found in the CSF. In a study of rats [76], intrastriatal ALA administration increased locomotor activity and induced seizures. The authors were unable to ascertain the involvement of GABA and suggested that an underlying glutamatergic mechanism induced convulsions.

Tryptophan and serotonin

During acute attacks of AIP, the blood level of tryptophan increases [20, 56]. This may be due to a lack of heme in liver cells inhibiting the breakdown of tryptophan by reducing the activity of hepatic tryptophan pyrrolase [161, 162], see Figure 2, **B +C**. Tryptophan is toxic in animal experiments and induces jerking movements in mice, rats and guinea pigs [140, 239]; these jerking movements can progress to tonic-clonic seizures. The ingestion of L-tryptophan in normal subjects is associated with CNS symptoms such as drowsiness, euphoria and nystagmus [233], severe abdominal pain and vomiting and even myoclonus and ocular oscillations [24]. In the brain, tryptophan is a substrate for serotonin formation [56]. The reported involuntary movements may be due to a central effect of serotonin [55, 140]. Porphyria carriers excrete large amounts of the serotonin breakdown product 5-HIAA in their urine during attacks [164].

The continued administration of tryptophan to rats with impaired hepatic tryptophan degradation and elevated levels of circulating tryptophan leads to structural changes in brain astrocytes and oligodendroglia and the degeneration of Purkinje cells and wasting axons [23, 58]. Similar neurohistological alterations have been reported in patients who had had acute porphyric attacks [28, 209].

The CNS effect of tryptophan may be due to the toxicity of the amino acid or to serotonin. Increased amounts of serotonin in the brain may account for some of the symptoms seen in porphyria, such as depression and anxiety [268], and emetic and nociceptive effects [4]. The activation of peripheral serotonin receptors results in the contraction or relaxation of intestinal smooth muscles, the facilitation of peristaltic reflexes, arrhythmias, hypertension and the modulation of cholinergic/purinergic transmission in the urinary bladder [114]. A role for serotonin in the pathogenesis of seizures is less likely [177].

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is present in all living organisms from the most primitive one-celled algae that evolved more than three billion years ago to *homo sapiens* and the molecular structure is identical in each organism (Reiter 2001). Lerner [157, 158] discovered the hormone and named it melatonin from *mela,* because the hormone lightens the cell that produces the pigment melanin, and *tonin,* because the hormone in humans is derived from the compound serotonin.

Melatonin is synthesised from tryptophan in the pineal gland. The pineal gland plays a crucial part in an external and internal rhythm-generating system, which involves the eye, the suprachiasmatic nucleus (SCN) and the pineal gland. The nerve cells of the SCN transmit chemical messages on a regular schedule, approximately twelve hours "on" and twelve hours "off". The SCN signals the pineal gland to produce a hormone, melatonin, that is secreted into the blood stream. The internal rhythm is synchronised via light with the external 24-hour day/night signal. The retina detects the light. Melatonin is primarily secreted by the pineal gland into the blood stream rather than into the cerebrospinal fluid [221].

Melatonin is synthesised from tryptophan in the pineal gland in several steps, see Figure 2, **C+E+ F**. The rate-limiting enzyme is N-acetyltransferase (NAT) [70, 141]. The activity of NAT is influenced by the environmental light-dark cycle. The production of melatonin decreases with age [126, 264]. The physiological secretion of melatonin measured in blood and urine reaches its peak when the person sleeps in darkness, between midnight and dawn [214]. In contrast, the urinary excretion of porphyrins does not display a circadian variation in healthy persons without AIP [68]. Melatonin is metabolised in the liver to 6-sulfatoxymelatonin, which is excreted in the urine.

Melatonin depresses CNS excitability [52, 216] and has an anti-convulsive effect in animal models and in humans [3, 82]. However, it is also suggested that melatonin may have a pro-convulsive effect [227]. A pro-convulsive effect may be in concert with the fact that subgroups of epileptic patients primarily experience seizures at night [128] when plasma melatonin levels are several times higher than during the day [18, 198, 200, 215].

Acute attacks of AIP may be accompanied by heme deficiency, which may cause an increase in the serum level of tryptophan [20, 56]. Even though melatonin is synthesised from tryptophan, a study of 12 women with MAIP has demonstrated low serum levels of melatonin despite high levels of blood tryptophan and serotonin [209].

As almost all anti-epileptic drugs, with the possible exception of sodium bromide [217], vigabatrin and gabapentin [246], may precipate and aggravate AIP attacks, the treatment of an AIP-gene carrier with epilepsy is difficult.

The objective of Paper II was to investigate whether AIP-gene carriers who had had epileptic seizures excreted significantly lower levels of melatonin in their urine compared with AIP relatives who had never had an epileptic seizure and, if so, in further studies, to assess melatonin treatment of epileptic seizures in AIP-gene carriers. In Study II, we found lower levels of melatonin excreted in the urine of AIP-gene carriers who had had epileptic seizures compared with AIP relatives without seizures. This may be in line with an anti-convulsive effect of melatonin in AIP-gene carriers. However, the number of AIP-gene carriers and epileptic seizures may be too small to draw any firm conclusions.

Summary of AIP and epileptic seizures

The prevalence of epileptic seizures in AIP-gene carriers is 3.7% in Sweden, substantially lower than previously reported but still higher than in the general population. Hyponatremia was demonstrated in half the carriers with seizures during an acute porphyric attack. As almost all anti-convulsive drugs may provoke attacks of acute porphyria, it is important to measure the levels of ALA and PBG before and after instituting AED treatment to determine whether seizures are due to the acute AIP attack itself or to other causes, such as drug treatment.

The exact mechanisms underlying epileptic seizures in AIP-gene carriers are not known, but it is likely that multiple mechanisms are in operation. We do not know whether the level of ALA in the CSF is sufficiently high to induce convulsions, or whether the level of excitatory amino acids such as glutamate is raised. In future studies, these levels in the CSF should be investigated in AIP-gene carriers during acute porphyric attacks and in remission.

AIP and diabetes mellitus, Paper III

The ample ingestion of carbohydrates reduces the excretion of porphyrin precursors in urine in experimental AIP [223]. Treating an acute attack with a high dose of carbohydrate may often significantly ameliorate symptoms and this may be due to the depression of the production of the rate-limiting enzyme ALAS 1 in the synthesis of heme [183] by glucose, see Figure 2. Heme deficiency, which is thought to precede attacks, causes increased levels of tryptophan in the liver and increased hepatic tryptophan inhibits the activity of phosphenol puruvate carboxykinase (PEPCK, Figure 2G), which is necessary for gluconeogenesis and glycogen formation. Intermediates proximal to PEPCK, such as lactate, puruvate, aspartate, malate and oxalacetate, increase several fold, whereas distal intermediates are depleted [207, 250]. In rats, tryptophan inhibits glycogen formation from the intermediates proximal to PEPCK but not from the intermediates distal to the block, such as glucose, fructose or glycerol [56]. Treatment with glucose presumably redresses the metabolic glucose imbalance [119, 183]. The clinical response to high carbohydrate intake varies, however [260].

A 57-year-old man with manifest AIP experienced a cessation of AIP attacks after a type 2 DM was diagnosed [271]. In our study (Paper III), 16 patients with AIP and DM were found. After the onset of their DM, no patient suffered attacks or any other AIP symptoms.

Why should higher blood glucose levels, as can be seen in DM, protect AIP-gene carriers from acute attacks of porphyria? The suppression of ALAS is unlikely as the sole reason, since ALAS is an intracellular enzyme and insulin deficiency is accompanied by low intracellular levels of glucose. It is possible to hypothesise that AIP-gene carriers may have a high intake of carbohydrates to treat AIP symptoms and thus trigger latent DM. This hypothesis is, however, also unlikely, since 50-70% of the AIP-gene carriers with DM in our study had never had any AIP symptoms. A third possibility is that high levels of blood glucose correct the abnormal glucose tolerance seen in acute porphyria by reducing the effects of changes in tryptophan metabolism [119, 183].

Proliferator-activated receptor γ co-activator α (PGC-1α) is a co-activator of nuclear and other

transcription factors [208] and controls mitochondrial biogenesis and oxidative metabolism in many tissues, such as skeletal muscle, heart and liver. In the liver, PGC-1α is induced during fasting and potently turns on the expression of the ALAS1 gene in hepatocytes. Insulin and glucose blunt the expression of PGC-1α, reducing ALAS1 expression [48, 121, 192].

The relatively high age of carriers with both AIP and DM makes it difficult to draw any conclusions regarding a protective effect by DM in AIP. After menopause, women seldom have acute attacks, whereas attacks are more commonly seen among men > 40 years of age. There is a need to pool available records on AIP and DM and large prospective studies would probably be necessary to elucidate the question of whether DM has a protective effect on attacks in AIP. The AIP-gene carriers with high levels of HbA1c traced in this study will be followed to see whether they have attacks of AIP in the future.

AIP and multiple sclerosis, Paper IV

As we had met two DNA-diagnosed AIP-gene carriers who also had suspected MS due to white-matter lesions seen on brain MR and/or OB in the CSF, we performed this study to screen for MS in a population of AIP-gene carriers.

One of the gene carriers, a non-smoking man, was born in 1951. He complained of paresthesia and dizziness in connection with a voluntary weight loss of 13 kg at the end of the 1980s. The diagnostic work-up disclosed high-signal, white-matter lesions on brain MR and one IgG band was detected in the CSF. During the course of the 1990s, he had attacks of paresthesia and limb weakness (not abdominal pain). During these attacks, the excretion of ALA and PBG in urine increased, while ALA and PBG in urine were within normal reference limits between attacks. This case is similar to two cases described by Kotze in 1999 [143]. They had MS and porphyria-like symptoms, such as limb weakness and sensory loss, with an increase in the excretion of porphyrins in the urine. These cases highlight some similarities between MS and acute porphyria. Both diseases may lead to limb weakness and autopsy may reveal similar changes [72, 99]. Kotze's cases were not diagnosed as a known type of porphyria. Atypical attacks of AIP with paresis and anaesthesia without abdominal pain have been described elsewhere [11].

Post-mortem studies of patients who had had attacks of acute porphyria have revealed demyelination in peripheral and autonomic nerves and focal perivascular demyelination in the CNS. Case reports have revealed reversible multi-focal lesions on brain MR during acute attacks of AIP [1, 39, 139, 147, 204, 253]. However, no previous study has investigated whether MAIP-gene carriers in remission have white-matter lesions (WML) on brain MR, or increased protein or OB in the CSF. The potential relationship between MS and AIP is important, as drugs may be used to reduce the risk of relapses in MS but not in AIP and as certain drugs should be avoided in AIP-gene carriers. It may be difficult to make treatment decisions for a patient with both MS and AIP, as it may be challenging to determine whether the actual symptoms are due to MS **or** AIP.

We found that four AIP-gene carriers (25%) had white-matter, high-signal T2W lesions, but only one subject had multiple lesions, while the other three subjects had fewer than ten scattered lesions and did not comply with the McDonald criteria for MR support of a diagnosis of MS [172].

Three subjects were between 58 and 60 years of age, while one was 35 years old.
The carrier with multiple high-signal T2W lesions and one of the carriers with fewer than ten white-matter lesions had a diagnosis of treated hypertension. Brain WML are seen secondary to a varity of pathological processes associated with many different myelin abnormalities which make the diagnostic investigation of WML challenging [85]. White-matter lesions on brain MR are seen in patients with MS [91], hypertensive small vessel disease (degenerative microangiopathy), inflammatory disorders [43], multiple infarcts and migraine [145]. There is also an increased prevalence of white-matter lesions with increasing age. One limitation of this study is that no control group was used to compare the result from the MR and lumbar puncture investigation. To our knowledge, no study has investigated OB in healthy volunteers (HV) and we felt it was unethical to perform lumbar puncture on HV who would not benefit in any way from the investigation.

None of our subjects with white-matter lesions had an established diagnosis of MS, vasculitis or migraine. Although it seems unlikely that the MR lesions are due to porphyria, this possibility cannot be entirely ruled out. Future studies could investigate AIP-gene carriers during attacks and in remission in a longitudinal design to elucidate whether white-matter lesions come and go due to attacks of AIP.

No AIP-gene carrier in Paper IV had OB in the CSF, making it unlikely that demyelinating lesions play a pivotal role in the pathogenesis of neurological symptoms and findings. This is an important finding, as many of the investigated subjects had had numerous attacks of AIP, including pareses. Latorre & Muñoz [155] found no cells in the CSF but increased protein levels in 67% of the investigated AIP-gene carriers during acute attacks. In our Study IV, we found increased protein levels in only one case. Increased protein levels are probably only seen during acute attacks of porphyria.

Clinical aspects of AIP in northern Sweden – a population-based study, Paper V

Remarkable progress in understanding the genetic and biochemical factors behind the disease has taken place during the last decade. However, for many years now, little interest has focused on the clinical issues of AIP. We therefore carried out this study to update our knowledge of AIP in order to improve the prevention, control and treatment of AIP and to be aware of potentially associated diseases.

We examined 356 AIP-gene carriers from the four northernmost counties in Sweden with regard to underlying genetic defects. We also described the clinical symptoms and severity of AIP attacks, as well as precipitating factors including alcohol and smoking habits, relevant laboratory data, concomitant diseases possibly related to AIP and the prevalence of long–term sick leave and disability pension.

We found that 42% of the AIP-gene carriers had MAIP and that the majority of these were women. The number of subjects with MAIP is higher than generally reported [11]. Hospital admission due to AIP attacks was reported by 75% of the gene carriers with MAIP. MAIP was more common in women compared with men; a 2:1 ratio was found, in accordance with other large series [7, 98, 137, 255, 257, 273]. Eight mutations were found in the study area in northern Sweden. The high prevalence of MAIP in our study could be due to the fact that 90% of the gene carriers had the W198X mutation, which has been shown to have high penetrance [14]. The participants in our study were well characterised and examined in detail, which might also contribute to the high percentage of AIP.

Symptoms of AIP were more severe in women when it came to the number and duration of attacks, hospital admission and early onset. The AIP attacks were most troublesome during their most reproductive period of life and only a minority reported attacks after menopause. On the other hand, men reported most troublesome attacks at > 40 years of age.
A surprisingly high percentage, 75% of the carriers with MAIP, reported a specific trigger factor for the attack. In women, menstruation was reported as the most common reason for porphyric attacks, while alcohol, physical and psychological strain, certain drugs and work environment were more commonly reported as trigger factors in men. Sex hormones play an important role in precipitating attacks in women, but the direct role of sex hormones is still not known. Pregnancy has previously been associated with AIP attacks, but not in our study or later reports from Finland [137] and South Africa [122].

In this study, 61% reported the daily use of drugs (MAIP: LAIP 79%: 43%). Pharmacological treatment was reported as a common trigger factor (20%) for AIP attacks and half the carriers with MAIP used drugs that have been classified as unsafe or probably not safe in AIP according to Swedish recommendations in 1999. The most common indications for pharmacological treatment were hypertension and psychiatric diseases. Our experience that most diuretics and ACE inhibitors did not appear to be porphyrinogenic has contributed to new recommendations (www.porphyria-europe.com).

We also found an association between smoking and frequent attacks. Smoking has been proposed as a trigger factor for AIP attacks, which could be explained by porphorinogenic factors in the cigarette smoke [159]. Only two carriers actively reported smoking as a trigger factor for attacks in our study, where 50% of the gene carriers were smokers or ex-smokers (56% MAIP, 45% LAIP).

Abdominal pain was the most commonly reported symptom in our study (86%), which is in accordance with other studies. Fatigue during and after acute attacks was also a common symptom (42%) in our study and, to our knowledge, this has not been reported before. Fatigue was a great problem for some AIP-gene carriers, as it could persist for a long time after the acute attack. Twenty-nine per cent of the gene carriers with MAIP reported psychiatric symptoms during AIP attacks (depression, anxiety, hallucination-psychosis). The relevance of psychiatric disorders apart from anxiety is still the subject of debate [49, 57, 75, 213]. Significantly higher levels of U-ALA and U-PBG were found in MAIP compared with LAIP-gene carriers. Notably, 38% of the gene carriers with LAIP and 72% of the carriers with MAIP in remission displayed levels of PBG in urine above the reference limit. The corresponding figures for U-ALA were 14% and 35%, respectively.

Elevated levels of ASAT and bile acids, adjusted for age and gender, indicate mild hepatopathy, which is reported by others [11, 137]. The levels of creatinine and creatinine clearance indicate a significant impairment in renal function in carriers with MAIP, in accordance with other studies [16, 137, 150].

A causal relationship between MAIP and SLE and antinuclear factor (ANA) has been proposed, but, according to our results, any such association appears to be coincidental. Hypertension and pain in the legs (not yet reported by others) were significantly associated with MAIP, adjusted for age and gender. No such association was found regarding stroke or myocardial infarction, even if hypertension was over-represented in MAIP, or in diabetes mellitus, epileptic seizures and psychiatric diseases. The same finding applied to kidney, liver

and cancer disorders in general, which comprised several different diseases under each disorder, i.e. kidney disorders included kidney stones, nephritis and others. However, hepatocellular cancer was highly over-represented, in accordance with other studies [6, 13, 29, 105, 112, 136, 160]. For this reason, an annual radiological screening of the liver is recommended for all AIP-gene carriers, aged 50-55 years or older.

Chronic AIP symptoms were reported by 16% of the women and 23% of the men, most of whom were smokers. The symptoms were usually unspecific, such as abdominal and muscle pain, weakness and fatigue, and no specific treatment is available. Increased levels of U-PBG may be a prerequisite in order to consider these symptoms AIP related.

Twenty per cent of the MAIP carriers were receiving disability pension, most of them were women and smokers. Previous studies have shown that gene carriers with MAIP have a poorer quality of life than in other forms of porphyria and the gene carriership produces life event consequences, such as unemployment, limitation of family size and being refused insurance [178]. Depression and anxiey are more common than in the general population [178].

The strengh of this retrospective population-based study is that it included nearly all the AIP-gene carriers > 18 years of age in the northern part of Sweden, almost half of all known carriers in Sweden, with a few drop-outs. The AIP-gene carriers in this study are well characterised and were examined by the same two doctors and nurse following a standardised protocol. The results of the study may therefore permit generalisation.

One problem in clinical investigations of AIP is that there is unfortunately no consensus for a definition of an AIP attack. In this study, a combination of a) positive heredity, b) symptom constellation attributable to, or prominent in, AIP attacks (not chronic symptoms), c) intermittent course of the symptoms, when applicable, d) levels of porphyrin precursors above the reference limit and e) no obvious cause of the symptoms was regarded as an AIP attack, assessed by two doctors experienced in AIP in Sweden.

One limitation of this study is the retrospective design; the number, duration and severity of attacks in the past may suffer from recall bias, as 50% of the carriers had had their last attack more than ten years ago.

In the future

Acute intermittent porphyria is not a harmless disease. Despite remarkable progress in our understanding of the genetic and biochemical factors behind the disease, measures such as early diagnosis and counselling regarding precipitating factors should be taken in order to reduce conversion to MAIP. We estimate that a reduction from the present 40-50% AIP-gene carriers with MAIP (in northern Sweden) to 10-20% would be possible to achieve using these measures. Despite these measures, a certain percentage of AIP-gene carriers will suffer from acute attacks, mostly women due to endogenic factors. There is therefore a certain need to develop new treatments. The use of recombinant human porphobilinogen deaminase (rhPBGD) in acute attacks of AIP produced reduced levels of PBG but not of ALA and had no effect on the clinical symptoms during an attack. Approaches to reduce ALA in liver cells should be considered in future drug development. A correction of the biochemical defect in AIP using viral and non-viral gene delivery has been shown in fibroblasts and, in future gene therapy, the use of this technique may also be relevant for AIP. Liver transplantation may be considered as a valid treatment for selected carriers with severe recurrent attacks and significant disabling neurological function.

The European Porphyria Initiative (www.porphyria-europe.com) was set up in order to compare experience between countries, to develop a common approach to the management of porphyrias and to facilitate international collaborative clinical research and development which will make it possible to study large populations with porphyria for the benefit of the carriers of these inherited diseases.

Conclusions

1.1 Epileptic seizures are less common in AIP-gene carriers than hitherto thought. The prevalence is only slightly higher than in the general population. However, the prevalence of seizures in gene carriers with MAIP is higher than in the general population. Sixty per cent had their seizures during acute attacks of AIP and half of them had hyponatremia.

1.2 Various types of seizure occur in AIP. Seizures may be generalised from the onset, or they may be partial becoming secondarily generalised.

2. AIP-gene carriers who had had epileptic seizures had a lower concentration of melatonin in their urine as compared to AIP relatives without a history of seizures. This may suggest a possible anti-convulsive effect of melatonin.

3. The prevalence of diabetes mellitus (DM) in AIP-gene carriers did not differ from the observed prevalence of DM among adults in Sweden. No AIP-gene carrier had any attacks of AIP after the diagnosis of DM. The question of whether DM may benefit AIP-gene carriers remains open.

4.1 A quarter of gene carriers with MAIP and LAIP had white-matter, high-signal lesions on brain MR. Multiple aetiologies such as hypertension, other vascular encephalopathy and ageing may contribute to the lesions.

4.2 Elevated levels of protein in the CSF were rare in AIP-gene carriers and oligoclonal bands were not found in the CSF. We found no evidence of an association between MS and AIP. A diagnosis of AIP should, however, be considered in patients with suspected MS or Guillan-Barre.

5.1 In the AIP population in northern Sweden, comprising almost half the Swedish AIP population, eight mutations in the PBGD gene were present, with a predominance of the W198X mutation. About 40% of the gene carriers had MAIP; two thirds were women.

5.2 Women were more severely stricken by attacks in terms of the number of attacks, hospital admission and early onset. The AIP attacks were more troublesome during their main reproductive period in life and were uncommon after menopause. Men, on the other hand, reported most troublesome attacks after the age of 40 years.

5.3 The most common symptoms reported during attacks were severe abdominal pain, constipation, vomiting, muscle pain, psychiatric symptoms, pareses and sensory impairment. We also report fatigue as a common symptom lasting for days after the attack. Chronic AIP symptoms were reported by 18%.

5.4 Specific trigger factors for the AIP attack were frequently reported. Sex differences were found; menstruation was reported as the most important precipitating factor in women, while alcohol, physical and psychological strain, certain drugs and work environment were more commonly reported in men

5.5 Drugs as trigger factors for AIP attacks were reported by 20% of women and men. Half the MAIP-gene carriers used drugs classified as unsafe or probably not safe in 1999 without

any problems, mainly diuretics, ACE inhibitors and SSRI, which are now considered safe or probably safe.

5.6 We found an association between smoking and a high frequency of AIP attacks.

5.7 Hypertension and pain in the legs were associated with MAIP, adjusted for age and gender, but not with stroke, myocardial infarction, diabetes mellitus, epileptic seizures, kidney or liver disease. Hepatocellular cancer was highly over-represented, but this did not apply to other forms of cancer.

5.8 Elevated levels of ASAT, bile acids, creatinine, U-ALA and U-PBG and reduced levels of creatinine clearance were associated with MAIP. Positive antinuclear factor was associated with low creatinine clearance but not with AIP and the relationship between AIP and SLE therefore appears to be coincidental.

5.9 In a follow-up study in 2001, 20% of the MAIP-gene carriers reported receiving a disability pension due to AIP. The majority of them were women and smokers.

5.10 AIP is not a harmless disease. Measures should be taken to obtain an early diagnosis, together with counselling regarding precipitating factors in order to reduce the conversion to MAIP and reduce the number and severity of attacks. Attacks should be treated promptly and attention should be paid to associated diseases, including screening and treatment. These approaches are important in order to improve the life and prognosis of AIP-gene carriers.

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