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The Genetics of Acute Hepatic Porphyria and Emerging Molecular Therapies for Clinical Management

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ABSTRACT

Acute hepatic porphyria (AHP) is a disease process resulting from a defect in the heme production pathway in the liver. Symptoms are due to the buildup of porphyrin precursors, which contributes to the damage of a variety of organ systems. There are different types of AHP depending on which enzymatic gene is affected and to what extent. The disease typically presents with nonspecific symptoms, so a high degree of suspicion is usually required to make the diagnosis of AHP. The current standard of management typically involves the avoidance of precipitating factors and acute attacks are mainly managed with supportive treatment and administration of intravenous hemin. However, new molecular therapies are emerging, which are demonstrating efficacy in decreasing the frequency and severity of symptomatic attacks by downregulating inappropriately expressed genes using siRNA or replacing deficient proteins by introducing mRNA. This paper explores the pathophysiology, diagnosis, and management of AHP and highlights emerging therapies designed to combat this disease process.

Keywords: AHP; Genetics, Molecular Therapy

1 Introduction

Heme is an important cofactor for many proteins. It is famously known for its contribution to the oxygen carrying properties of red blood cells when it joins with globin proteins to form hemoglobin. It is also associated in myoglobin, cytochromes in the electron transport chain, catalase, endothelial NOS, and other protein complexes. Heme is synthesized by a series of enzymatic reactions occurring in the mitochondria and cytosol of cells. Many tissues in the body produce heme; however, its biosynthesis primarily occurs in erythroblasts and hepatocytes in the bone marrow and liver, respectively. The first step of the synthetic pathway involves combining succinyl CoA and glycine to form delta-aminolevulinic acid (ALA), which is catalyzed by ALA synthase (ALAS). This is the rate limiting step, committing the substrates to the pathway. There are two types of ALAS: ALAS1 and ALAS2, which are unique to hepatic and erythroid cells, respectively. The rest of the pathway is essentially the same in both tissues, with the next step being the condensation of two ALA molecules to porphobilinogen (PBG) via ALA dehydratase (ALAD). Next, four PBGs are linked by PBG deaminase (PBGD) to form hydroxymethylbilane, which then undergoes cyclization by uroporphyrinogen III synthase (UROS) to form the first porphyrin product of the pathway, uroporphyrinogen. Uroporphyrinogen decarboxylase (UROD) then catalyzes production of

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coproporphyrin, which is then converted to protoporphyrinogen via coproporphyrinogen oxidase (CPOX). Protoporphyrinogen is decarboxylated by protoporphyrinogen oxidase (PPOX) to produce protoporphyrin. And finally, iron is added to protoporphyrin by ferrochelatase to produce heme as a final product (Fig. 1).

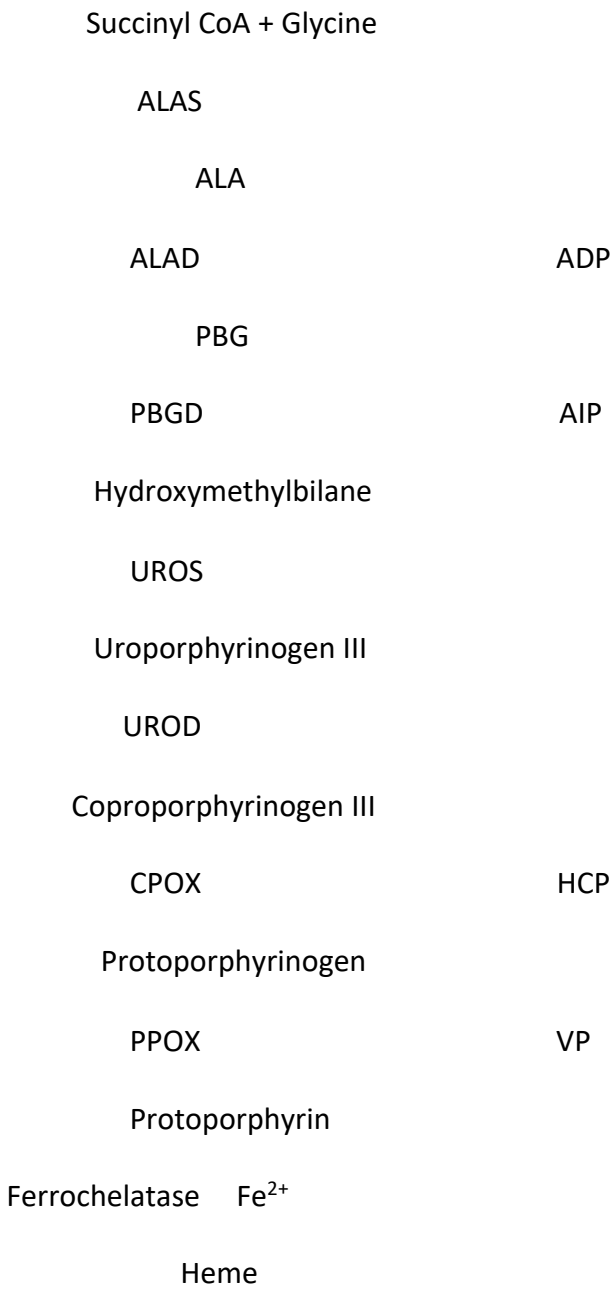

Figure 1. Heme biosynthetic pathway, enzymes involved, and associated AHP.

Porphyrias are metabolic errors caused by a defect in the heme synthetic pathway, which can occur at any of the eight enzymatic steps in the process. This defect leads to a buildup of porphyrin precursors, often causing damage to the nervous system and/ or skin.[1] Acute hepatic porphyria (AHP) is the disease process resulting from a defect in the heme production pathway in the liver. It is caused by inherited genetic mutations conferring dysfunction of a key synthetic heme enzyme in hepatocytes. There are four types of AHPs: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) and ALAD deficient porphyria (ADP). AIP, HCP and VP are autosomal dominant (AD) disorders and ADP is inherited autosomal recessively (AR).[2,3] The affected enzymes contributing to each of the respective AHPs

are: PBGD, CPOX, PPOX, and ALAD (Fig. 1). These deficiencies can predispose patients to triggering factors (eg. drugs, hormones, smoking, alcohol, nutritional factors, etc), leading to the accumulation of ALA and PBG following exposure. These heme precursors are neurotoxic; therefore, AHP typically manifests as episodic acute neurovisceral symptoms.[2,3]

Acute hepatic porphyrias can be a difficult group of diseases to diagnose since the symptoms resemble other, more common, conditions. But it is important to keep this group of disorders in mind when examining a patient as it can be easily screened for via urinalysis, identified by elevated ALA and PBG in urine.[4] AHP can cause a number of acute and long-term complications, so prompt treatment of acute episodes and diligent monitoring are required for management and prevention of long term neurologic, hepatic or renal sequala. Current treatment methods involve intravenous carbohydrate infusion and heme replacement.[4] This has proven to be effective for acute attacks of AHP; however, adverse events and tachyphylaxis has been an issue in some cases.[5] New molecular therapies involving the use of small interfering RNA (siRNA) and messenger RNA (mRNA) are being investigated and are demonstrating promise for future clinical management.[2]

2 Genetics and Pathophysiology of AHP

As previously mentioned, three of the four AHPs are the result of an inherited AD mutation (AIP, HCP, VP) and one is attained by AR inheritance (ADP). A variety of mutations have been identified to affect the genes coding for the involved enzyme. It may be missense, nonsense, insertion, deletion, frame shift, or another type.[6-8] The enzyme functionality varies slightly depending on the type of mutation, but most result in 50% activity compared to normal phenotype due to one non-functioning and one functioning allele (in AD disease or heterozygous AR trait).[3] With that being said, 90% of those carrying the AD mutation never experience an attack.[2] Therefore, importance should also be placed on the environmental, metabolic, and genetic cofactors involved in the pathogenesis of symptomatic AHP. For example, drugs that are metabolized by cytochrome P450 can increase heme turnover, which causes increased heme biosynthesis through negative feedback of ALAS, resulting in the production and accumulation of more heme precursors.[6] Additionally, attacks of AHP have also been associated with the menstrual cycle, possibly due to fluctuations of estrogen and progesterone.[2]

AIP is the most common AHP, and the disease subtype that presents with the most severe symptoms.[2] It arises due to a mutation of PBGD, leading to decreased formation of hydroxymethylbilane and a buildup of PBG and ALA with low heme formation. The accumulation of these heme precursors is neurotoxic and the decreased heme formation leads to continued activity of ALAS1 due to negative feedback. This perpetuates the production of PBG and ALA, eventually leading to symptomatic AIP.[2] There are over 420 distinct mutations to the PBGD gene identified to date, which is located on chromosome 11q23.3.[9] Fu[6] studied the effects of point mutations on the crystal structure of PBGD to understand how these mutations affect protein function and discovered that mutations resulting in symptomatic disease tend to occur at residues in substrate binding sites.

VP is caused by a mutation to the PPOX gene, located on chromosome 1q22-23, and is the most prevalent AHP in South Africa.[1,7,10] Over 177 distinct mutations have been identified for VP; however, the R59W missense mutation is found in approximately 96% of South Africans with AHP due to an established founder effect.[7] This subtype of AHP results in neurologic symptoms similar to AIP and HCE, but cutaneous lesions often predominate.[10]

HCP is caused by a mutation to the CPOX gene, located on chromosome 3q12.[1,8] Over 65 mutations have been identified and this is the least common of the autosomal dominant porphyrias.[3,8] Clinical presentation is mostly indistinguishable AIP, unless photosensitized cutaneous lesions appear; however, this only occurs in about 30% of cases.[8]

ADP is an autosomal recessive subtype of AHP and is very rare. Mutation to the ALAD gene (chromosome 9q32) results in less than 3% of normal enzyme activity and the accumulation of ALA only, leading to the neurovisceral symptoms of AHP.[2]

Other gene products interacting with the heme synthetic pathway have also been identified, suggesting that AHP attacks may have a polygenic cause. Of note, peroxisome proliferator-activated receptor alpha (PPARA) has a direct influence on cytochrome P450 regulation. Therefore, a PPARA mutation may cause increased heme turnover and subsequent ALAS1 upregulation, contributing to symptomatic attacks.[6] Additionally, a symptom of AIP is hyperlipidemia and it is well known that PPARA is involved in lipolysis, again suggesting a connection between the two systems. Another gene of interest is estrogen-related receptor alpha (ERRSA). ESSRA can affect the normal expression of ALAS1, which provides an explanation as to why AHP attacks can sometimes be tied into menstrual cycle timing.[6]

Overall, the pathogenesis of each condition is the same: decreased enzyme activity leads to low heme production, activation of ALAS1, and buildup of heme precursor molecules, which elicit neurovisceral symptoms with accompanying cutaneous lesions in some cases. Attacks are largely triggered by an insult that stresses the heme biosynthetic pathway, resulting in the excess production of heme precursors. It is important to note that there is still approximately 50% of residual enzyme activity in all three AD subtypes, which is enough for some individuals to never experience an attack of AHP.

3 Clinical Presentation and Diagnosis

The signs and symptoms of AHP are relatively nonspecific; however, a typical clinical picture of a patient with an acute attack of AHP can still be assembled. Attacks primarily occur in females aged 15-45 who present with diffuse lower abdominal and/or back pain, which has been increasing in severity over the past few days.[3] The patient will likely complain of nausea, vomiting, constipation and weakness. Generalized seizures may occur in 10-20% of cases.[3] In VP, the patient will also likely have cutaneous lesions on sun-exposed areas, typically presenting as blisters, which heal leaving persistent hypo- and hyperpigmented patches.[10] It may also be revealed in the history that the patient has presented to the emergency department before with similar symptoms but a specific diagnosis was unable to be reached.[3] On clinical exam, the patient will be afebrile with no leukocytosis.[2,3] They may be tachycardic, hypertensive, and likely be hyponatremic.[2,3] It is possible to observe mildly elevated transaminases and dark urine as well.[2,3] And abdominal imaging will be negative.[3]

When AHP is suspected, further biochemical testing is conducted to confirm the suspicion. A patient with AHP will have elevated urine levels of ALA and PBG.[1-4] These markers will stay elevated for a long period in AIP, but not in HCP or VP.[1,4] However, stool porphyrins are often markedly elevated in those subtypes so a diagnosis is still possible in the absence of an ongoing acute attack.[1,2] In the autosomal recessive ADP, urinalysis will show large amounts of ALA but not PBG, since ALAD is required to convert ALA to PBG.[4] After AHP is confirmed through biochemical testing, genetic testing is undergone to determine the appropriate subtype of AHP.[4] Here, the genes of PBGD, CPOX, and PPOX are sequenced to identify the deficient protein (ADP would be confirmed via biochemical testing alone, indicated by the normal PBG levels).[4] Identifying the specific mutation is important because it enables testing of at-risk family members.[4] Unfortunately, there are no strong genotype-phenotype correlations to predict disease severity or prognosis.[4]

4 Current Management

Management of acute attacks involves first discontinuing the potential precipitants. Next, initial treatment involves administration of high carbohydrates intake orally if possible, otherwise it is administered intravenously.[2,3] This works to initially downregulate ALAS1 while the mainstay treatment, intravenous hemin, is being administered. Intravenous hemin replaces the heme deficiency and more effectively decreases ALAS1 activity; however, symptom improvement generally takes about four days to be observed.[2,3] During this time, opioid analgesics are usually required to manage the severe pain along with anti-emetics for controlling nausea and vomiting.[2,3]

Patients with AHP should undergo hepatic, renal, and neurological evaluations, especially those patients who experience multiple recurrent attacks.[2,4] Liver function tests should be obtained for all patients at

baseline.[2,4] There is a higher risk of hepatocellular carcinoma (HCC) in AHP patients regardless of symptomatic status.[2-4] Those with evidence of chronic liver disease should undergo annual screening for cirrhosis and HCC with ultrasound and serum alpha fetoprotein starting at age 50.[2-4] A metabolic panel and estimated glomerular filtration rate should also be obtained at baseline and monitored annually for all symptomatic patients because AHP carries a high prevalence of chronic kidney disease.[2-4] Recurrent attacks can be complicated by chronic neuropathy, typically axonal motor neuropathy causing paresis.[2,4] Patients experiencing frequent attacks may also develop chronic neuropathic pain and/or sensory deficits.[2,4] Therefore, nerve conduction studies and/or electromyography conducted by a neurologist is recommended in patients with neurological deficits.[4] Finally, psychiatric management may be necessary in symptomatic patients. Recurrent attacks can impose a significant strain on physical and emotional well-being due to chronic pain and disability. This can lead to a significant reduction on quality of life and precipitate anxiety, depression, and even psychotic manifestations.[10] Therefore, psychiatric evaluation and treatment may also be beneficial (or necessary) for long-term management of symptomatic AHP.

If patients begin to undergo severe, disabling attacks with intractable disease despite prophylactic hemin, a liver transplant may be considered. This operation is curable but is a treatment of last resort due to high morbidity and mortality.[2-4]

5 Emerging Therapies

Current treatment methods have stood the test of time and are proven to be efficacious. However, there have been some reported issues with adverse effects and tachyphylaxis, which limits the treatment potential for some patients.[5] New targeted molecular therapies have been emerging in recent years, which are demonstrating efficacy in decreasing the frequency and severity of symptomatic attacks and may offer a solution to preventing the need for end-stage liver (and/or kidney) transplant.

The AHPs all present similarly and follow a common pathogenesis. The main factor contributing to liver heme precursor overproduction is ALAS1. When a trigger results in decreased levels of heme, the biosynthetic pathway is activated. ALAS1 is the rate limiting step, so once it combines succinyl CoA and glycine to form ALA, the pathway is committed. If the downstream enzymes are not functioning optimally, the heme precursors build up and cause damage due to their neurotoxic properties. Therefore, ALAS is a prime candidate target for emerging AHP molecular therapies. One approach involves downregulating ALAS1 levels by introducing hepatocyte-specific siRNA.[11,12] Here, the ALAS1 mRNA transcript is bound by a complementary strand of ALAS1 siRNA. The newly formed double stranded RNA can now enter the RNA-induced silencing complex (RISC), where it is cleaved and degraded.[2] This ultimately prevents translation of the ALAS1 enzyme, leading do decreased levels of ALA and downregulation of the heme biosynthesis.[11,12] A specific drug that has shown good outcomes in phase I and IIa clinical trials in humans is Givosiran (Alnylam Pharmaceuticals, Cambridge, MA, USA). This drug is an ALAS1-specific siRNA linked to N-acetylgalactosamine to facilitate site-specific delivery to hepatocytes via the asialoglycoprotein receptor (a liver-specific glycoprotein receptor).[11] Monthly administration of Givosiran resulted in significant reduction in urine and plasma ALAS1 mRNA, decreased levels of urine ALA and PBG, and reduced attacks requiring hospitalisation, urgent healthcare visit, or hemin administration.[13] Clinical trials are still underway to study the pharmacodynamic, pharmacokinetic, safety, and efficacy of Givosiran. One particular complication being investigated is the potential reduction in the liver's detoxification capacity from long-term down-regulation of ALAS1.[13]

Conversely, other emerging treatment methods involve replacing the enzyme activity that has been lost. This has been experimented through: 1. Recombinant adeno-associated virus (rAAV)-mediated transfer of human cDNA coding for PBGD[14,15]; and 2. Introduction of wild-type PBGD mRNA.[16] Unzu[14] demonstrated the potential for increasing protein expression by introducing a copy of normal PBG cDNA into hepatocytes by way of rAAV in a proof of concept study involving mice models. Unfortunately, phase I clinical trials failed to reduce porphyrin precursor levels despite documented safety and overall positive clinical outcomes in most patients.[15] Gene therapy using rAAVs has been approved in some conditions

such as inherited retinal diseases.[17] However, gene therapy using viral vectors can produce a humoral-mediated immune response, which can cause wide variability in the expression of the therapeutic protein and result in variable treatment efficacy.[18] This may be what led to the disappointing clinical trials encountered by D'Avola.[15] Fortunatelty, administration of mRNA may prove to be a simpler, more effective method of increasing PBGD levels. Human PBGD mRNA can be packaged in lipid nanoparticles and selectively internalised by hepatocytes through the interaction between apolipoprotein E on the nanoparticle and the low-density lipoprotein receptor on the hepatocyte.[18] The mRNA is then released into the cytoplasm where it is translated into functional human PBGD protein. Studies on animal models have demonstrated over-expression of PBGD, dose-dependent reduction of ALA and PBG levels, and effective prevention of AHP attack when it is administered during the prodromal phase.[13] The mRNA method has an advantage over the transgene method because it does not have to counteract an immune response. It also requires less steps to produce functional protein after its administration, since mRNA can be translated directly in the cytoplasm. Conversely, cDNA first needs to be integrated into the nucleus for transcription, then transported to the cytoplasm for translation; a process that would require more complex genetic engineering to ensure the appropriate signal molecules are properly expressed. Clinical trials to introduce mRNA as a treatment for AHP are expected to start in the near future.[2,13]

One other emerging molecular therapy approach has been investigated by Bustad[9] in a proof of concept study using chaperone proteins to stabilize wild type (WT) PBGD in a mouse model with AIP. PBGD is a very stable enzyme, but it loses some conformational stability when it is bound to a substrate or undergoing catalysis.[19] The researchers identified compounds that further stabilize WT PBGD, which should theoretically provide modestly enhanced activity to the enzyme. This additional activity may be adequate to avoid an AIP attack, regardless of the mutation.[9] The authors propose pharmaceutical chaperone therapy as a potential oral treatment that can be used prophylactically or as an intervention during an acute attack pending further clinical research.

6 Conclusion

AHP is a disease caused by a mutation in enzymes involved in heme biosynthesis. Decreased function leads to buildup of neurotoxic heme precursors that may lead to an attack of neurovisceral symptoms. Although symptomatic AHP may be rare, the prevalence of AHPs may be higher than previously thought. There are three AD subtypes of AHP and one AR, but the clinical presentation is similar between each. The key is to recognize the symptoms of an acute attack, followed by biochemical analysis and genetic testing to confirm the presence of AHP and reveal the subtype. The current treatment of IV hemin transfusion along with high carbohydrates and pain relief has stood the test of time thus far; however, exciting new targeted therapies are emerging. They work by either downregulating ALAS1 using siRNA or by replacing the deficient protein by introducing the normal mRNA to hepatocytes. These therapies are undergoing clinical trials and show promise for the future management of AHPs. Perhaps the molecular therapies can even work alongside genetic screening to prophylactically treat affected individuals in order to prevent ever experiencing a symptomatic attack.

7 Competing Interests

The author certifies that they have no affiliation with or involvement in any organization with financial or non-financial interest in the subject matter discussed in this manuscript.

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