

# Recent advances in the epidemiology and genetics of acute intermittent porphyria

Liyan Ma<sup>1</sup>, Yu Tian<sup>1</sup>, Chenxing Peng<sup>1</sup>, Yiran Zhang<sup>2</sup>, Songyun Zhang<sup>1,\*</sup>

<sup>1</sup>Department of Endocrinology, The second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China;

<sup>2</sup>School of First Clinical Medical College, Southern Medical University, Guangzhou, Guangdong, China.

**SUMMARY** Acute intermittent porphyria (AIP) is a dominant inherited disorder with a low penetrance that is caused by mutations in the gene coding for hydroxymethylbilane synthase (HMBS). Information about the epidemiology and molecular genetic features of this rare disorder is crucial to clinical research, and particularly to the evaluation of new treatments. Variations in the prevalence and penetrance of AIP in various studies may due to the different inclusion criteria and methods of assessment. Here, the prevalence and penetrance of AIP are analyzed systematically, and the genetic traits of different populations and findings regarding the genotype-phenotype correlation are summarized. In addition, quite a few studies have indicated that AIP susceptibility was affected by other factors, such as modifying genes. Findings regarding possible modifying genes are documented here, helping to reveal the pathogenesis of and treatments for AIP. The status of research on AIP in China reveals the lack of epidemiological and genetic studies of the Chinese population, a situation that needs to be promptly remedied.

**Keywords** acute intermittent porphyria, prevalence, penetrance, genetic traits, genotype-phenotype correlation, modifying genes

## 1. Introduction

Porphyrias are a group of metabolic diseases that result from a specific abnormality in one of the eight enzymes of the heme biosynthetic pathway (1,2). In general, porphyrias are classified either as acute porphyrias or cutaneous porphyrias based on their clinical presentation or as hepatic and erythropoietic porphyrias based on the tissue where heme precursors are overproduced (3).

Acute intermittent porphyria (AIP, OMIM#176000), the most common and severe form of acute hepatic porphyrias (4), is an inherited metabolic disease that exhibits an autosomal dominant pattern of inheritance caused by partial deficiencies in hydroxymethylbilane synthase (HMBS; EC 2.5.1.61), the third enzyme in heme biosynthesis (5). AIP has significant molecular genetic heterogeneity and low penetrance (6). It leads to accumulation of upstream metabolites  $\delta$ -aminolevulinic acid (ALA) and porphobilinogen (PBG), which induce toxicity to the neurologic system, and then trigger episodic, acute neurovisceral symptoms that can even be life-threatening (7-11). Studies of the prevalence, penetrance, and molecular genetic traits of AIP are crucial to its early diagnosis and rational management. Thanks to the rapid development of next-generation

sequencing (NGS) technology, genetic sequencing has been widely used to detect HMBS gene mutations (12,13). Some studies based on genetic testing have revealed that the prevalence of HMBS variants was substantially underestimated, with extremely low penetrance in the general population but higher penetrance in families with AIP (14,15).

Findings regarding modifying genes and the correlation between genotype-phenotype warrant more attention in recent studies on AIP. Although a genotype-phenotype correlation has not been identified, certain mutations may be relevant to penetrance or the severity of clinical manifestations. Some modifying genes that have been identified in recent years are reviewed here, including the PEPT2 gene, PPARA gene, cytochrome P450 gene, and ABCB6 gene.

In China, concern about AIP has increased, but most studies on AIP are various case reports and small series. The prevalence, penetrance, and genetic traits of Chinese patients with AIP are still unclear and need to be fully evaluated.

## 2. Prevalence and penetrance of AIP

AIP is an autosomal dominant metabolic disorder with

a variable prevalence among different countries (16). Because it is a rare disease with multiple phenotypes, its prevalence is difficult to evaluate (17). Therefore, information about the prevalence of AIP is most often based on estimates. Variations in the prevalence and penetrance of AIP in various studies are probably due to different inclusion criteria and methods of assessment (18). Information is classified in Table 1 as the prevalence of symptomatic AIP, the prevalence of pathogenetic *HMBS* mutations (including data on the general population and families with AIP), and penetrance (including data on the general population and families with AIP).

Much of the information on AIP comes from a 3-year prospective study of newly diagnosed symptomatic patients with AIP in 11 European countries, and the annual incidence of symptomatic AIP was reported to be 0.13 per million. Its prevalence, which was calculated based on the incidence and mean disease duration, was 5.9 per million in Europe (19). Moreover, the numbers were similar in all countries except Sweden (19). The rate of recurrence, which was 3-5%, was also been estimated in that study. In addition, the sliding prevalence of symptomatic AIP indicates the importance of improved management and educational strategies (19-22). Similarly, a 60-year retrospective study in Finland revealed a decrease in patients with active AIP (23,24). In light of the number of patients with AIP referred to a French facility, the prevalence of overt AIP was

estimated to be 7.6 per million, which was in line with the aforementioned figure of 5.9 per million (25).

Due to founder effects, the prevalence of AIP was markedly higher in some regions, such as 17.7 per million in southeastern Spain (26) and 23 per million in Sweden (19). In northern Sweden, it was even 192 per million (27). Information from Argentina and Western Australia may less credible because no gene sequencing was performed (28,29).

Although symptomatic patients with AIP are quite rare, some findings based on population have indicated that the prevalence of pathogenetic *HMBS* mutations was higher than previously estimated. A study based on Caucasians indicated that the prevalence of pathogenetic *HMBS* mutations was 1/1,782 (14). This result was consistent with the findings of two studies in France, which estimated the prevalence of mutations in the *HMBS* gene to be 1/1,675 to 1/1,299 (15,25).

Thus, this information indicates a marked discrepancy in the estimated prevalence of *HMBS* mutations and the occurrence of acute attacks in patients with AIP, which means that its penetrance is very low. As of the current point in time, penetrance is estimated to be approximately 20-50% in families with AIP but only ~1% in the general population, with the exception of 23% in the Swedish and 42% in the Northern Swedish (14,19,25,27,31,32). This finding strongly suggests that other factors act as a catalyst for AIP attacks, such as modifying genes and environmental factors.

**Table 1. The estimated prevalence and penetrance of AIP**

Country (region) or Population	Prevalence of symptomatic AIP (case per million inhabitants)	Prevalence of pathogenetic <i>HMBS</i> mutations		Penetrance (%)		Ref.
		in the general population	in families with AIP (%)	in the general population	in families with AIP	
Caucasian		1/1,782		< 1		14
Europe	5.9					19
France	7.6	1/1,299		0.5-1	22.9	25
		1/1,675				15
	5.5					19
Sweden	23			23		19
		1/10,000				30
Northern Sweden		1/1,000				30
	192	1/2,000		42		27
Switzerland	10-15		50		52	31
	9.9					19
Southeastern Spain	17.7					26
Spain	6.3				52	19
Finland			47		35	32
	5.9					19
Norway	6.3					19
Northern Italy	5.0					19
Poland	7.2					19
UK	7.2					19
Netherlands	8.1					19
Russia			59		57	58
Argentina*	8				40	28
Western Australia*	24				29	29

\*No gene sequencing was performed in the study

### 3. Genetics of AIP

#### 3.1. Molecular bases

Genetically, AIP is an autosomal dominant disorder resulting from mutations in the *HMBS* gene (33), which is located at the chromosomal region 11q24.1-24.2 (GRCh38.p11:119,084,003-119,094,417). There are two isoforms of HMBS, the housekeeping and the erythroid isoforms (34). The transcript including exons 1 and 3-15 is directed by housekeeping promoter located in the 5' flanking region upstream of exon 1, and the transcript containing exons 2-15 is produced by an erythroid-specific promoter located in a region 3 kb downstream of intron 1 (35-37). Most patients with AIP carry mutations in exons 3-15, which affect not only the housekeeping but also the erythroid isoforms of HMBS, which are the classic form of AIP. When mutations occur within or close to the coding region of exon 1, the activity of HMBS in erythroid cells is normal (38-40). Regulatory gene defects in the 5' -promoter regions of the *HMBS* gene have been found in patients with AIP (41). To date, no variants have been found in the erythroid-specific promoter or in exon 2 (42).

#### 3.2. Molecular genetic features

Thus far, over 500 different mutations in the *HMBS* gene have been identified (Human Gene Mutation Database HGMD, <http://www.hgmd.cf.ac.uk/ac/index.php>). There are 31 CpG dinucleotides, at which most of the *de novo* events in human genes appear, in the 1,086 base-pair coding sequence of *HMBS* (14,43). Because of oxidative deamination of methylated cytosines, the CpG dinucleotides are believed to be hypermutable (44). Most of the mutations resulting in AIP are private or present only in a few unrelated families, a fact that highlights the molecular genetic heterogeneity of AIP (45-53,73). Nevertheless, a few mutations are relatively common either due to CpG dinucleotide mutational hotspots in the gene (43), such as the mutations encoding p.R173W and p.R167Q, or due to a founder effect, such as *HMBS* P.G111R in Argentina and p.W198X in Sweden (54,55). (Table 2 shows the founder effect mutations causing AIP among different populations).

Since AIP is an autosomal dominant disease, no sex differences in gene carriers are expected (60,61), but

all studies have found that acute attacks affect females much more frequently than males (19,54,59,60,62-64). A recent study in Finland found a high penetrance of 41% for AIP acute attacks and 50% for all acute manifestations associated with AIP in female patients (32). Similar findings have been gleaned from families with the founder mutation p.W198X in northern Sweden (27,65). These studies emphasize that female hormones trigger AIP attacks (23,66,67).

#### 3.3. Genotype-phenotype correlation

To date, no clear genotype-phenotype correlation for AIP has been determined (24,63,68). However, some studies have indicated that some mutations may be related to a higher penetrance and/or more severe clinical manifestations, such as p.W198X, c.1073delA, and p.R26C (32,69), while some variants may be associated with a lower penetrance and/or milder manifestations, including p.R167W, p.R225G, and c.G33T(24,32,69). Interestingly, conflicting findings were obtained from studies on P.R173W; a study in Finland reported a lower penetrance but studies in Northern Sweden and Spain reported a higher penetrance (16,32,69). Although a study in Finland found similar genotypes, penetrance differed greatly among families (32). This shows that the phenotype of AIP is not determined by the *HMBS* genotype alone but that other factors such as modifying genes and the environment also play a vital role in the pathogenesis of AIP attacks (6,24,25,32,70,71). Moreover, protein function analysis and bioinformatic tools have been used to identify a genotype-phenotype correlation. Some mutations in the *HMBS* gene including p.R116W, p.R173W, p.R149X, p.Q217H, p.G218R, p.A219P, and p.A330P have been predicted to lead to severe clinical manifestations (72). The correlation between genotype and phenotype is still a topic of interest in research on AIP.

#### 3.4. Modifying genes

The low penetrance of AIP and the significant difference between the penetrance found in families with AIP and that found in the general population indicate that AIP susceptibility is affected by the inheritance of *HMBS* gene mutation as well as other genetic or environmental factors. A hypothesis has been put forth that AIP

**Table 2. Founder effect mutations causing AIP among different populations**

Population	Mutation (cDNA)	Mutation (Amino Acid)	Exon	Ref.
Dutch	c.346C > T	P.R116W	8	51
Canadian (Nova Scotia)	c.517C > T	P.R173W	10	56
Swedish	c.593G > A	P.W198X	10	55
Swiss (German-speaking)	c.848G > A	P.W283X	14	31
Argentinean	c.331G > A	P.G111R	7	54
Spanish (Murcia, southeastern region of Spain)	c.669_698del		12	57
Russian	c.53delT	p.Met18ArgfsX3	3	58

inheritance does not follow the classical autosomal dominant pattern but an oligogenic or polygenic inheritance pattern with environmental modifiers (25,58,74,75).

Many attempts using various approaches have been made to search for those modifying genes in order to both further understand the pathogenesis of AIP and to identify reliable therapeutic targets.

#### 3.4.1. *PEPT2* gene

Genetic variation in human peptide transporter 2 (*PEPT2*, also known as *SLC15A2*) has been identified as an aspect in modulating the severity of renal and neurologic impairment (6,76-78). ALA is reabsorbed in the proximal tubules by *PEPT2* variants. Variants of *PEPT2* have different affinities for ALA: *PEPT2\*1* has a higher affinity for ALA while *PEPT2\*2* has a lower affinity (79,80). Thus, more significant neurotoxicity may occur in *PEPT2\*2* carriers because they have lower ALA brain efflux (81-83). That said, *PEPT2\*1* with a higher affinity for ALA is associated with an increased risk of renal disease (6,76).

#### 3.4.2. Cytochrome P450 gene

The enzyme 5-aminolevulinic acid synthase (ALAS) 1 catalyzes the rate-limiting step in the production of heme and is regulated by heme through a negative feedback loop (84). Many of the substances that induce cytochromes (CYPs) in the endoplasmic reticulum of the liver can also result in increased hepatic ALAS1 activity (85,86).

Studies have indicated that the hepatic cytochrome P450 gene may play a role in AIP attacks (87). A study found that *Cyp2c40*, *Cyp2c68*, and *Cyp2c69* were upregulated in mice with induced AIP but downregulated in wild-type mice. These genes are respectively equivalent to *CYP2C8*, *CYP2C9*, and *CYP2C19*, which are the primary human CYP450 enzymes involved in drug metabolism. *Cyp21a1*, the homolog of human *CYP21A2* or *CYP17A1* (-37), is a crucial enzyme in corticosteroid and sex hormone synthesis; *Cyp21a1* is downregulated in mice with induced AIP but upregulated in wild-type mice (88,89). A study based on the population in the Spanish region of Murcia revealed parallel findings: the alleles *CYP2D6\*4* and *\*5* may prevent acute attacks in patients with AIP while *CYP2D6* may constitute a penetrance-modifying gene (26).

In short, the cytochrome P450 gene may be related to the pathogenesis of AIP, but further studies are needed to confirm this hypothesis.

#### 3.4.3. *PPARA* gene

Peroxisome proliferator-activated receptor alpha

(*PPARα*) is a transcription factor belonging to the nuclear receptor superfamily, and *ALAS1* has been identified as a target (90). In general, nuclear receptors regulate transcription through interactions with coactivator or corepressor molecules (91-95). Binding of agonists to *PPARα* leads to an enhanced binding of co-activator proteins, such as the proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) (96,97). PGC-1 $\alpha$ , which acts as a bridge from the activated *PPARs* to the basal transcriptional machinery (90), activates the *ALAS1* promoter by coactivating the nuclear respiratory factor-1 (NRF-1) and the forkhead box O1 (FOXO1), both of which directly bind to the *ALAS1* promoter (98).

Recently, a study based on protein functional analysis indicated that the *PPARA* gene has obvious functional overlap with *ALAS1* (72). That study also indicated that transcription of *CYP2C8* and *CYP3A4* is directly regulated by the *PPARA* gene (99), which is similar to previous findings that genes in CYP classes 1-3 are specifically regulated by *PPARA* in humans (100-103). Thus, mutations in *PPARA* may affect the heme biosynthesis pathway by regulating the cytochrome P450 system and eventually lead to AIP attacks (104).

#### 3.4.4. Some genes regulating the nervous system

Currently, a few defects in genes regulating the nervous system (*UNC13A*, *ALG8*, *FBXO38*, *AGRN*, *DOK7*, and *SCN4A*) have been detected in a study in Russia (58). The latter three genes are related to congenital myasthenic syndrome (CMS), whose symptoms bear a remarkable resemblance to neurologic features of AIP attacks. Thus, the combination of a mutation in the *HMBS* gene with defects in genes regulating the nervous system may play a key role in triggering acute AIP attacks and prompting the development of specific symptomatic traits (58). However, this assumption needs to be verified in future studies.

#### 3.4.5. *ABCB6* gene

Elevated porphyrins are a hallmark of various types of porphyria. *ABCB6*, a type of porphyrin transporter, has been investigated as a modulator of porphyria severity through deep sequencing and biochemical analysis. A study found that patients with severe porphyria have variant alleles in the *ABCB6* gene (105). Plasma membrane *ABCB6* exports multiple disease-related porphyrins. Functional studies have revealed that most of these *ABCB6* variants are poorly expressed and also dysfunctional. Therefore, *ABCB6* is presumably a genetic modifier of porphyria that alleviates its severity by expelling porphyrins.

Nevertheless, most reports of modifying genes are only preliminary findings, and further clinical studies are needed to verify their reliability.



#### 4. Status of research on AIP in China

For a long time, China's healthcare system has paid less attention to rare diseases, including AIP (106,107). An early study in the Mainland found a high rate of AIP misdiagnosis (33). Over a decade ago, a study involving 24 unrelated Chinese patients with AIP in Taiwan found no genotype/phenotype correlations, but the spectrum of *HMBS* gene mutations detected in these patients with AIP coincided with those observed in patients of other ethnic origins (61).

In recent years, this rare disorder has garnered attention from Chinese physicians, and small series of and case reports on AIP, and especially reports of novel mutations, have increased in China, indicating that AIP may not be as "rare" as was previously assumed (108-114). In addition, some studies have analyzed clinical features of Chinese patients with AIP, such as posterior reversible encephalopathy syndrome (110,115-118). Peking Union Medical College Hospital first described how acute attacks affected the quality of life and psychological state of patients with AIP in Northern China (119).

Unfortunately, there are as of yet no studies on the prevalence, penetrance, and genetic traits of Chinese patients with AIP. Findings from Western countries are most likely inapplicable to the Chinese population due to the obvious differences in ethnic characteristics. Therefore, data on Chinese patients need to be evaluated in order to promptly identifying patients with *HMBS* mutations that have not yet suffered attacks, to properly manage patients, and to improve prognosis.

#### 5. Conclusion

AIP is an inherited metabolic disease that exhibits an autosomal dominant pattern of inheritance caused by partial deficiencies in *HMBS*. In Europe, the estimated prevalence of AIP was 5.9 cases per million population, while the prevalence of *HMBS* variants was 1/1,299~1/1,782, with penetrance estimated at 20-50% in families with AIP but only ~1% in the general population. AIP has marked molecular genetic heterogeneity. No clear correlation between genotype and phenotype has been confirmed for AIP, although studies have reported that some mutations may be relevant to its penetrance or the severity of clinical manifestations. Consequently, the prevailing view is that other factors, such as modifying genes, may play an essential role in AIP attacks. Studies have identified some likely modifying genes, but most of those studies are just initial studies based on trials or predictions. Further clinical studies are needed to verify their reliability. Recently, studies on AIP have increased in China, but the prevalence, penetrance, and genetic traits of AIP in Chinese patients are unclear. Studies are urgently needed to reveal those aspects.

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\*Address correspondence to:

Songyun Zhang, Endocrinology, The Second Hospital of Hebei medical University, Shijiazhuang 050000, Hebei, China. E-mail: zsy2020@hebmu.edu.cn

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