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International Porphyria Molecular Diagnostic Collaborative: an evidence-based database of verified pathogenic and benign variants for the porphyrias

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Abstract

With the advent of precision and genomic medicine, a critical issue is whether a disease gene variant is pathogenic or benign. Such is the case for the three autosomal dominant acute hepatic porphyrias (AHPs), including acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria, each resulting from the half-normal enzymatic activities of hydroxymethylbilane synthase, coproporphyrinogen oxidase, and protoporphyrinogen oxidase, respectively. To date, there is no public database that documents the likely pathogenicity of variants causing the porphyrias, and more specifically, the AHPs with biochemically and clinically verified information. Therefore, an international collaborative with the European Porphyria Network and the National Institutes of Health/National Center for Advancing Translational Sciences/National Institute of Diabetes and Digestive and Kidney Diseases (NIH/NCATS/NIDDK)-sponsored Porphyrias Consortium of porphyria diagnostic experts is establishing an online database that will collate biochemical and clinical evidence verifying the pathogenicity of the published and newly identified variants in the AHP-causing genes. The overall goal of the International Porphyria Molecular Diagnostic Collaborative is to determine the pathogenic and benign variants for all eight porphyrias. Here we describe the overall objectives and the initial efforts to validate pathogenic and benign variants in the respective heme biosynthetic genes causing the AHPs.

Keywords

acute hepatic porphyrias; database; pathologic variants; benign variants; variant validation

INTRODUCTION

The acute hepatic porphyrias

The acute hepatic porphyrias (AHPs) include four inherited genetic disorders of heme biosynthesis, which are characterized by acute life-threatening attacks of nonspecific neurologic symptoms.^{1–4} Each of the AHPs results from the deficient activity of a distinct enzyme in the heme biosynthetic pathway. Table 1 summarizes the specific enzymatic deficiencies that cause the four AHPs: the three autosomal dominant disorders, acute

intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), and the very rare autosomal recessive aminolevulinic acid dehydratase deficient porphyria (ADP). The autosomal dominant AHPs are distinct from other porphyrias because of their common overproduction of the porphyrin precursors, 5-aminolevulinic acid (ALA) and porphobilinogen (PBG), which are understood to mediate the acute attack symptoms through a neurotoxic mechanism.^{1,2,5} The major manifestations of the acute porphyrias are therefore neurologic, including excruciating abdominal pain, a progressive peripheral neuropathy, and mental disturbances (e.g., confusion, fatigue, insomnia, etc.).^{1,2,4} The AHP neurovisceral attacks generally occur after puberty, are more common in women, and are currently most efficiently treated by intravenously restoring hepatic heme homeostasis with human hemin (Panhematin, Recordati Rare Diseases) or heme arginate (Normosang, Orphan Europe Recordati Group). Photosensitive skin lesions are not a feature of AIP, whereas patients with VP or HCP can present with either cutaneous or neurovisceral symptoms or both.

Although the specific enzyme and gene defects for each disorder were identified decades ago,¹ diagnosis of these disorders still presents formidable challenges because their symptoms and signs may mimic other, more common conditions and the correct diagnosis depends on specialized laboratory services. Notably, delaying diagnosis and treatment of acute porphyric attacks can be fatal or cause long-term permanent neurological damage.

Biochemical and molecular diagnosis of the AHPs

A porphyria diagnosis depends on laboratory investigations to demonstrate the pattern of heme precursor accumulation and excretion specific for each type of porphyria. Acute attacks of porphyria are diagnosed by showing marked elevation of urinary PBG and ALA, the diagnostic “gold standard” of these diseases, with analysis of plasma and fecal porphyrins necessary to differentiate among the different AHPs.^{6–8} Elevated levels of the plasma porphyrin precursors can also be detected by more sensitive mass spectrometry methods, although these are not yet widely available.⁹ With the isolation and characterization of the heme biosynthetic genes and identification of the pathogenic variants causing these diseases,^{1,2} genetic analyses provide confirmatory diagnoses and identify the specific pathogenic AHP gene variants, enabling the screening of asymptomatic at-risk family members (i.e., “latent heterozygotes”) who should be counseled to avoid factors that are known to precipitate acute attacks.^{1,2,4,7,10}

RATIONALE FOR AN INTERNATIONAL DIAGNOSTIC DATABASE OF VERIFIED PATHOGENIC AND BENIGN VARIANTS

The advent of genetic-based diagnoses of the AHPs presents additional challenges, specifically in determining whether a gene variant is pathogenic or benign. This is a common issue in gene-based diagnostics, as benign variants are common.¹¹ In the past, studies often included a few hundred “normal individuals” as controls to estimate the frequency of newly identified variants, with the assumption that purported pathogenic variants for rare AHPs did not occur in the populations studied. More recently, the availability of exome/genome databases (e.g., gnomAD¹²) with tens of thousands of alleles

from different ethnic and demographic groups has assisted in the confirmation or reclassification of previously diagnosed “pathogenic” variants as either pathogenic or more common benign variants. Table 2 summarizes the reported numbers of the missense, nonsense, and consensus splice-site variants and their allele frequencies (AF) in various ethnic and demographic populations in the gnomAD database¹² for the three autosomal dominant AHP-causing genes: hydroxymethylbilane synthase (*HMBS*), coproporphyrinogen oxidase (*CPOX*), and protoporphyrinogen oxidase (*PPOX*).

The Human Gene Mutation Database

To date, the Human Gene Mutation Database (HGMD) has been the most reliable resource for variant data in the AHP genes.¹³ However, porphyria diagnostic laboratories worldwide frequently identify additional novel pathogenic or benign variants that are not published, and as such are not available in the HGMD.

In addition, the HGMD curation policy includes most published variants,¹³ although some variants’ pathogenicity may be questionable. In the case of AIP, of the total 423 *HMBS* variants in version 2019.1, 141 (33%) were missense variants, many of which were reported as disease-causing without sufficient supportive evidence for verification. Several of those initially reported as pathogenic missense variants were subsequently reclassified as “questionably pathogenic” based on subsequent studies of allele frequency, in vitro expression, and in silico prediction analyses.^{12,14}

Intronic variants that alter splicing can also be difficult to assess unless there is clear clinical and biochemical documentation of their pathogenicity. For example, *HMBS* c.613–31A>G, also known as IVS10–31A>G, was initially reported as a pathogenic variant,¹⁵ but subsequently found to be very common in individuals of African or Afro-Caribbean descent.^{12,16} Although HGMD currently classifies c.613–31A>G as questionably pathogenic,¹³ recent data in the United States indicate that c.613–31A>G heterozygotes or homozygotes have a benign *HMBS* polymorphism (gnomAD frequency in Africans: 0.43, Latinos: 0.021, and Caucasians: 0.0023). If it were pathogenic, all homozygotes would be expected to have infantile- or juvenile-onset of homozygous dominant AIP, which is a severe neurodegenerative disorder with early demise.¹⁷ Further support for its benign status is the finding of individuals with a known *HMBS* pathogenic variant and markedly elevated ALA and/or PBG, who were also heterozygous or homozygous for c.613–31A>G, but without severe early-onset symptoms (Table 3). Therefore, there is a need to establish a collaborative database to collect and verify previously reported, as well as currently unreported, AHP pathogenic and benign variants identified in expert porphyria diagnostic laboratories.

Direct-to-consumer testing and variant interpretation services

Another important development in support of a verified database of pathogenic variants is the recent availability of relatively inexpensive direct-to-consumer (DTC) genetic testing. These companies (e.g., 23andMe, AncestryDNA, MyHeritage DNA, FamilyTreeDNA) typically use relatively inexpensive chip-based assays of ~700,000 single-nucleotide polymorphisms (SNPs) to assess ancestry, and determine a very limited number of disease-causing variants, if any. In fact, the US Food and Drug Administration limits the type of

health-related information that DTC companies can market. Recently, individuals who have negative biochemical test results for the AHPs have obtained their underlying DTC genotype data and used third-party companies to interrogate the SNPs in the heme biosynthetic genes. Most of these SNPs are intronic, and the few exonic alterations are typically benign common polymorphisms that do not cause porphyria, including the *CPOX4* variant (rs1131857, NM_000097.5:c.814>C) that reduces enzyme activity, but not sufficiently to cause HCP. In particular, these SNPs are also limited in number, with fewer than ten pathogenic variants screened for the *HMBS*, *CPOX*, and *PPOX* genes whose messenger RNA (mRNA) transcripts are over 1000 nucleotides. The DTC test analyses are therefore not designed to diagnose the porphyrias. In contrast, certified diagnostic laboratories sequence the entire coding sequence, the exon–intron boundaries, and the upstream promoter (5′) and downstream (3′) regions of each heme biosynthetic gene, and can identify all pathogenic and benign variants by Sanger sequencing, or by RNA or multiple ligation-dependent probe amplification (MLPA) analysis.

Importantly, the inaccuracy of DTC gene testing and misinterpretation of the underlying sequence data were highlighted in a recent article¹⁸ and editorial titled “Attention: direct-to-consumer patrons: proceed with caution.”¹⁹ The authors call attention to the fact that often the DTC test results were inaccurate as they were not confirmed by a certified diagnostic laboratory. Moreover, many SNPs that were confirmed were actually benign. These findings further support the need for a public database of verified pathogenic variants that is curated by disease-specific experts.

ACMG and AMP guidelines for sequence variant classification

To address the problem of sequence variant classification, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) produced guidelines that take into account population, computational, functional, and segregation data.²⁰ This provides a framework to enable classification of variants as “pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” or “benign.” It has been noted that more focused guidance regarding the classification in specific genes is required as the applicability of certain criteria may vary by disease and gene.^{21,22} This supports the need to establish a disease-specific criteria and public database providing up-to-date information that will allow porphyria specialists to make informed decisions on the classification of variants in the AHP genes. The guidelines will continue to develop so that the reclassification of variants will be ongoing. Classification within the database will therefore be advisory and it will remain the responsibility of individual reporting laboratories to confirm the classification.

Rationale for a database of validated porphyria variants

The importance of human variant databases for identifying pathogenic and likely pathogenic variants causing specific genetic disorders has been the subject of several previous publications (e.g., refs.^{23–25}). Efforts have primarily focused on the assessment of variants that have been identified by exome and genome sequencing or by targeted sequencing of specific genes in patients with Mendelian or complex traits.²⁶ Variant databases such as ClinVar (www.ncbi.nlm.nih.gov/clinvar) have been established to document the validity of

gene–disease associations, and in particular, the pathogenicity of variants within genes to inform accurate diagnosis and guide patient care. For ClinVar, Clinical Domain Working Groups were established by the Clinical Genome Resource Consortium (ClinGen) of the National Human Genome Research Institute in conjunction with the National Center for Biotechnology Information to provide a public resource for diagnostic laboratories, researchers, and clinicians to collect and share genomic data, to ultimately determine the pathogenicity of specific variants identified with genome/exome and targeted sequencing by research investigators and commercial laboratories.²⁷

Clearly, for a given disease or disease group, validation of a variant's pathogenicity can optimally be assessed by disease experts working together with disease-specific diagnostic laboratories. Here, an international group of porphyria experts has established a consensus statement for the validation of pathogenic variants in the heme biosynthetic genes causing the AHPs. In addition, we outline the efforts of the International Porphyrrias Molecular Diagnostic Consortium to verify the published and currently unreported variants detected by expert porphyria laboratories in patients with the eight major porphyrias based on their diagnostic biochemical findings.

FORMATION OF THE INTERNATIONAL PORPHYRIA MOLECULAR DIAGNOSTIC COLLABORATIVE

At the 2017 International Congress on Porphyrins and Porphyrrias (ICPP) in Bordeaux, France, one of the authors (R.J.D.) proposed an International Collaborative to bring together experts in the biochemical and molecular diagnosis of the porphyrias to establish porphyria-specific evidence criteria and a public database of the validated pathogenic and benign variants in the heme biosynthesis genes, as well as an updated diagnostic consensus by international experts. Genetic alterations that cause porphyrias will be classified as pathogenic or likely pathogenic variants, whereas genetic alterations that have high allele frequency or that lack supportive biochemical and in vitro evidence will be classified as benign or likely benign variants. Genetic alterations with inconclusive evidence for pathogenicity will be classified as variants of unknown significance (VUS). Table 4 provides some examples of the evidence criteria for variant classification.

The database will validate as many of the published genetic alterations as possible for each porphyria based on de-identified clinical and biochemical data. In addition, benign variants identified by gene sequencing or genomic/exomic sequencing databases can be verified by high enzyme activity levels when expressed in vitro, in silico predictive algorithms, and/or relatively high frequencies in genomic/exomic databases. The database will be regularly updated with new pathogenic and benign variants identified by the participating expert laboratories. The International Collaborative will leverage participants' cumulative clinical expertise and biochemical data to rigorously assess each variant's pathogenicity. To pilot this International Collaborative, the initial goal will focus on collating pathogenic and benign variants in the genes causing the three autosomal dominant AHPs.

Porphyria experts from countries worldwide indicated their support of the International Collaborative, including the European Porphyria Network (EPNET) and the US Porphyrrias

Consortium. To date, EPNET and Porphyrrias Consortium members have established the following objectives for the International Collaborative: (1) establish a database of validated porphyria pathogenic variants, initially for the three autosomal dominant AHPs; (2) determine qualifying criteria for expert contributing diagnostic laboratories; (3) determine the validation requirements for each porphyria; and (4) enroll qualified porphyria diagnostic laboratories.

Database information

The database will provide two main features related to the diagnosis of the porphyrias: (1) a genetic variant search tool that will list validated pathogenic and benign variants classified according to current guidelines, and (2) a submission form to facilitate the contribution of new variants. The variant search tool will allow users to search for a gene alteration and provide information that supports its pathogenic or benign determination or its undetermined status. The electronic submission form provides the opportunity to submit novel genetic variants, which will be incorporated into the database after curation.

The database will follow the latest recommendations from the Human Genome Variation Society (HGVS) for the sequence nomenclature²⁸ but will also include “historical/legacy nomenclature” (e.g., IVS for intronic variants). The description is generally in reference to the respective standard sequence in the RefSeq collection (e.g., for *HMBS*, NM_000190.3, for *CPOX*, NM_000097.5, and for *PPOX*, NM_000309.4) or the stable Locus Reference Genomic identifier (e.g., for *HMBS*, LRG_1076, and for *CPOX*, LRG_1077). For alterations that are in the coding region, additional descriptions at the protein level will be provided, including the exon number and the protein reference ID (e.g., for *HMBS*, NP_000181.2; for *CPOX*, NP_000088.3; and for *PPOX*, NP_000300.1). If a variant has been reported in the literature, hyperlinks to the publications will be available for users to find additional information about the variant. For a pathogenic variant, clinical and/or biochemical information associated with the variant, together with additional data from in vitro assays, will be presented. For variants that have been identified in multiple probands, individual clinical, biochemical, and in vitro data will be summarized.

For variant data submission, the database will require, wherever possible, that the submitter provide de-identified clinical (i.e., cyclic, multiple, or sporadic attacks) and biochemical information (i.e., urinary and/or plasma ALA and/or PBG concentrations, fecal and plasma porphyrin data) to document the variant’s pathogenicity, or classification as benign or likely benign. Additional data from other analyses such as in vitro assays (e.g., enzyme assay, luciferase activity, or reverse transcription polymerase chain reaction [RT-PCR] studies), or in silico prediction algorithms will be recorded.

Criteria for contributing laboratories

The database aims to collect variant data submitted from expert diagnostic laboratories worldwide. Therefore, it is important to ensure that contributing laboratories perform genetic testing under an internationally recognized standard (ISO), such as ISO 15189 (ref.⁸). All contributing laboratories will be expected to participate in the quality assurance program of the European Molecular Genetics Quality Network (EMQN) and in the EPNET External

Quality Assessment Scheme for the porphyrias or other similar quality assurance programs. At this writing, the Collaborative is in the process of establishing the full criteria for contributing laboratories.

Genetic variant validation

The major activity of the International Collaborative will be to validate the pathogenicity of variants in the AHP-causing genes using clinical, biochemical, and other supporting data (Table 4) in line with current guidelines.²⁰

The gold standard for validating the pathogenicity of an AHP gene alteration for patients presenting with acute symptoms is the demonstration of markedly elevated urinary and/or plasma ALA and PBG concentrations when the patient is symptomatic,⁶ positive concentrations being at least fourfold greater than the upper limit of normal values for a given laboratory. In the above situation, and in VP or HCP patients presenting with skin lesions only, and where urine and plasma PBG and ALA are likely normal, unequivocal biochemical confirmation will rely on plasma and fecal porphyrin analysis to distinguish among the AHPs.^{8,29} Porphyrin biochemistry diagnostic of a specific AHP can be used within the Association for Clinical Genomic Science (ACGS) guidelines as strong evidence toward pathogenicity, as it is an aspect of the disease that is measurable and is pathognomonic of a defect in one of the AHPs.^{21,22}

It is important to note that a major focus of validation will be directed toward variants that are challenging to distinguish if they are pathogenic or benign. These variants primarily include missense and nonconsensus splice-site alterations, other intronic changes that can cause alternative splicing, and in-frame deletions or insertions that delete or insert amino acids without changing the rest of the encoded amino acid sequence.

METHODS FOR VARIANT VALIDATION

Porphyrin precursor measurements

When a patient is clinically suspected of having an acute neurovisceral attack, the first-line diagnostic test is an urgent spot urinary ALA and/or PBG determination, which should be normalized to the urine creatinine. A normal urinary PBG or near normal ALA and/or PBG excretion before treatment with human hemin or heme arginate excludes the diagnosis of an AHP in symptomatic new patients. In AIP patients, PBG and ALA may decrease after an attack but often remain somewhat elevated for years,³⁰ while in HCP and VP, PBG and ALA excretion may decline more rapidly. A proportion of AHP patients may also have constantly elevated ALA and PBG levels in the absence of symptoms and are termed chronic high excretors.^{30,31}

Since precursor levels during an attack are markedly elevated, preanalytical factors (e.g., exposure to light, lack of refrigeration) and/or shipping conditions (e.g., unfrozen, prolonged transit time) are unlikely to reduce the high levels of excretion into the normal range. However, with spot samples it is important to normalize values to the urine creatinine concentration. A subsequent measurement of total fecal porphyrins, the fecal coproporphyrin isomer III:I ratio, and the fluorescence emission spectrum of plasma

porphyrins will distinguish among VP, HCP, and AIP.^{29,32} Table 5 summarizes key porphyrin biochemical findings characteristic of active acute AHPs.

Enzyme assays

Measurements of erythrocyte HMBS activity have been used in the past to help confirm the diagnosis of AIP.³³ For HCP and VP, the leukocyte enzyme assays from blood samples are technically challenging and are not widely used.^{34–36} A major issue with the enzyme assays is the significant overlap of HMBS, CPOX, or PPOX enzymatic activity between high heterozygote and low normal ranges, which can lead to inconclusive results.^{37–43} HMBS activity is affected by the average age of the red cells in the collected sample, being higher in younger cells. In addition, for the diagnosis of AIP, *HMBS* variants in the codons of exon 1 and in intron 1 that alter normal splicing of the housekeeping transcript will markedly decrease the activity of the housekeeping HMBS isozyme (for review, see ref.⁴⁴) but will not alter the normal expression of the erythroid-specific isozyme, thereby leading to missed diagnoses if used in isolation.^{43,45,46}

In vitro expression of missense variants and in-frame deletions and insertions helps classify these variants identified in patients or in exome/genome databases (e.g., ref.¹²). In vitro expression of questionable missense variants can confirm pathogenicity when the enzyme activity is markedly deficient (<10%) and benign variants when the enzyme activity is >50%. Most deleterious variants showed very low or no residual activity;⁴⁷ nevertheless, there is no consensus as to what the cutoff is for a few variants with activity between 10% and 40% of the wildtype expressed activity. For variants with significant residual activity, it is useful to determine the stability of the expressed enzyme, for example, by heat inactivation analysis.¹⁴

RNA analysis

Routine Sanger sequencing should identify >98% of *HMBS*, *CPOX*, *PPOX*, or *ALAD* variants in AHP patients.²⁹ However, “cryptic” variants do occur rarely, in which the alterations are intronic, creating an alternative splice site, or occur in the promoter region altering expression. mRNA analysis, MLPA, or expression reporter assays (e.g., luciferase reporter assays) can be used to determine the effects of such variants.

In silico analysis

In silico predictive programs can also aid in the determination of pathogenicity for novel genetic variants. Although individual programs use different sets of principles for their prediction, many include sequence conservation and biophysical and biochemical properties of the amino acids as part of their criteria. It should be noted that current prediction accuracy plateaus at approximately 80% (ref.⁴⁸) and many programs have low specificity, resulting in overprediction of genetic variants as pathogenic.⁴⁹ As such these programs cannot be used alone.

CONCLUSIONS

The AHPs are rare disorders of heme biosynthesis. Accurate diagnosis of an acute porphyria presenting with an acute attack, or cutaneous symptoms in HCP or VP, depends on biochemical testing to confirm active porphyria by demonstrating abnormal porphyrin biochemistry consistent with the specific AHP. Genetic testing is now an established part of managing the families of patients with an AHP diagnosis worldwide. However, predictive genetic testing of at-risk family members requires identification of the symptomatic proband's specific pathogenic variant. As we move into the era of genomic medicine, genetic testing-based diagnostics will become an ever more common practice and will require disease-specific databases to assist in distinguishing pathogenic from benign variants. Thus, the International Porphyria Molecular Diagnostic Collaborative will provide the combined expertise of an international group of experts to facilitate accurate diagnosis for the porphyria community.

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Table 1

Characteristics of the four acute hepatic porphyrias

Disease	OMIM identifiers	Inheritance	Defective gene	Chromosomal location	Genomic coordinates	Known variants ^a
Acute intermittent porphyria (AIP)	176000	AD	Hydroxymethylbilane synthase (<i>HMB5</i>)	11 q23.2	chr1: 119,084,871–119,093,549	423
Hereditary coproporphyria (HCP)	121300	AD	Coproporphyrinogen oxidase (<i>CPOX</i>)	3q11.2	chr3: 98,568,448–98,593,684	73
Variegate porphyria (VP)	176200	AD	Protoporphyrinogen oxidase (<i>PPOX</i>)	1 q23.3, 6p22.2	chr1: 161,165,728–161,178,277	185
ALA-dehydratase deficient porphyria (ADP)	612740	AR	Aminolevulinatase dehydratase (<i>ALAD</i>)	9q32	chr9: 113,386,312–113,401,338	12

AD autosomal dominant, AR autosomal recessive.

^a Human Gene Mutation Database (HGMD Professional 2019.1).

Allele frequency distribution of missense, nonsense, and consensus splice-site variants in the *HMBS*, *CPOX*, and *PPOX* genes in various populations^a**Table 2**

Heme biosynthetic gene	Caucasians	Africans	Latinos	East Asians	South Asians
<i>HMBS</i>					
Missense					
0.4–0.1%	1	3	3	0	1
0.1–0.01%	7	11	8	7	9
0.01–0.002%	34	18	41	20	26
0.002–0.0007%	68	0	0	0	0
Nonsense					
0.01–0.002%	1	0	0	0	0
Consensus splice-site					
0.1–0.01%	0	0	0	0	1
0.01–0.002%	0	1	0	0	0
0.002–0.0007%	3	0	0	0	0
<i>CPOX</i>					
Missense					
45–1%	3	4	3	2	3
1–0.1%	0	3	2	3	4
0.1–0.01%	8	23	14	17	9
0.01–0.002%	31	23	15	21	31
0.002–0.0007%	53	0	22	0	0
Nonsense					
0.1–0.01%	0	1	0	0	0
0.01–0.002%	0	0	3	0	1
0.002–0.0007%	1	0	0	0	0
Consensus splice-site					
1–0.1%	0	0	0	0	1
0.01–0.002%	1	0	0	0	0
<i>PPOX</i>					
Missense					

Heme biosynthetic gene	Caucasians	Africans	Latinos	East Asians	South Asians
7-1%	1	1	1	1	1
1-0.1%	1	4	3	1	2
0.1-0.01%	3	17	7	13	8
0.01-0.002%	24	25	30	15	55
0.002-0.0007%	91	0	0	0	0
Nonsense					
0.1-0.01%	1	2	1	0	0
0.01-0.002%	2	1	1	1	2
0.002-0.0007%	6	0	0	0	0
Consensus splice-site					
0.002-0.0007%	1	0	0	0	0

^aData reported in Genome Aggregation Database (gnomAD v2.1.1, gnomad.broadinstitute.org).

Table 3

Identification of heterozygotes and homozygotes with the additional c.613–31A>G (IVS10–31A>G) variant among 273 probands tested for *HMB*S variants

<i>HMB</i> S variant ^a	Zygosity	c.613–31A>G (IVS10–31A>G)		Africans		Hispanics		Caucasians	
		N	%	N	%	N	%	N	%
With a pathogenic									
8	Heterozygotes	5	62	3	37	0	0	0	0
3	Homozygotes	2	67	0	0	1	33		
Total 11		7	63	3	27	1	9		
Without a pathogenic									
26	Heterozygotes	11	42	5	19	6	23		
1	Homozygotes	1	100	0	0	0	0		
Total 27		12	44	5	19	6	22		

^aPathogenic *HMB*S variants in AIP patients who are heterozygous (p.Gly111Arg, p.Arg116Trp, p.Gly218Arg, p.Gln153His, p.Arg173Trp, p.Arg225Ter, p.Gln332Ter, c.985_996del12, c.613–2A>G, c.912+1G>A) or homozygous (p.Arg167Trp, p.Arg195His, c.613–1G>T, c.478delC) for c.613–31A>G.

Table 4

Examples of evidence criteria for variant classification

Classification	Examples of criteria (porphyria-specific interpretation)
Pathogenic variant	<ul style="list-style-type: none"> Null variant (nonsense, frameshift, canonical $\pm$ 1 or 2 splice sites, initiation codon, single or multiexon deletion) in one of the AHP genes Unequivocal porphyrin biochemical results confirming a defect in the porphyria gene containing the variant <p>OR</p> <ul style="list-style-type: none"> Missense, in-frame deletion/insertion, or noncanonical splice-site variants in one of the AHP genes Unequivocal porphyrin biochemical results confirming a defect in the porphyria gene containing the variant Low allele frequency (<0.1%) in all ethnic groups Located in a well-established functional domain without benign variation
Likely pathogenic variant	<ul style="list-style-type: none"> Equivocal porphyrin precursor biochemical results confirming a defect in the porphyria gene containing the variant Low allele frequency (<0.1%) in all ethnic/racial groups
Likely benign variant	<ul style="list-style-type: none"> Allele frequency greater than expected for AHP Multiple lines of computational evidence suggesting no impact on gene or gene product
Benign variant	<ul style="list-style-type: none"> Variant allele frequency (>0.1%) in the AHPs
Variant of unknown significance	<ul style="list-style-type: none"> A variant for which there is conflicting or insufficient evidence for classification

Table includes some examples of how the evidence criteria are used to classify variants depending on available evidence. Further details of criteria that may be needed in classifying variants can be found in the Association for Clinical Genomic Science (ACGS) guidelines.^{21,22}

AHP acute hepatic porphyria.

Key porphyrin biochemistry findings characteristic of active acute versus cutaneous presentation of AHPs

Table 5

Porphyria	During acute attacks		Cutaneous manifestations			
	Urine ALA/PBG	Total fecal porphyrins	FCR	Plasma scan ^a (nm)	Urine ALA/PBG	Total fecal porphyrins
AIP	Highly increased > 4-fold URL	±Normal	<1.5	615–620	Not applicable	
HCP	Highly increased > 4-fold URL	Highly increased (Coproporphyrin III)	>1.5	615–620	±Normal	Highly increased (Coproporphyrin III)
VP	Highly increased > 4-fold URL	Highly increased (Coproporphyrin III & Coproporphyrin III)	>1.5	625–628	±Normal	Highly increased (Coproporphyrin III & Coproporphyrin III)

ALA 5-aminolaevulinic acid, Coproporphyrin III isomer, FCR fecal coproporphyrin III:I isomer ratio, PBG porphobilinogen, Proto IX protoporphyrin IX, URL upper reference limit.

^aPlasma porphyrin fluorescence scan emission wavelength.