

World Journal of *Clinical Cases*

World J Clin Cases 2022 November 26; 10(33): 12066-12461



Contents

Thrice Monthly Volume 10 Number 33 November 26, 2022

MINIREVIEWS

- 12066** Review of risk factors, clinical manifestations, rapid diagnosis, and emergency treatment of neonatal perioperative pneumothorax
Zhang X, Zhang N, Ren YY

ORIGINAL ARTICLE

Clinical and Translational Research

- 12077** Integrative analysis of platelet-related genes for the prognosis of esophageal cancer
Du QC, Wang XY, Hu CK, Zhou L, Fu Z, Liu S, Wang J, Ma YY, Liu MY, Yu H
- 12089** Comprehensive analysis of the relationship between cuproptosis-related genes and esophageal cancer prognosis
Xu H, Du QC, Wang XY, Zhou L, Wang J, Ma YY, Liu MY, Yu H
- 12104** Molecular mechanisms of Baihedihuang decoction as a treatment for breast cancer related anxiety: A network pharmacology and molecular docking study
Li ZH, Yang GH, Wang F
- 12116** Single-cell RNA-sequencing combined with bulk RNA-sequencing analysis of peripheral blood reveals the characteristics and key immune cell genes of ulcerative colitis
Dai YC, Qiao D, Fang CY, Chen QQ, Que RY, Xiao TG, Zheng L, Wang LJ, Zhang YL

Retrospective Study

- 12136** Diagnosis and treatment of tubal endometriosis in women undergoing laparoscopy: A case series from a single hospital
Jiao HN, Song W, Feng WW, Liu H
- 12146** Different positive end expiratory pressure and tidal volume controls on lung protection and inflammatory factors during surgical anesthesia
Wang Y, Yang Y, Wang DM, Li J, Bao QT, Wang BB, Zhu SJ, Zou L
- 12156** Transarterial chemoembolization combined with radiofrequency ablation in the treatment of large hepatocellular carcinoma with stage C
Sun SS, Li WD, Chen JL
- 12164** Coexistence of anaplastic lymphoma kinase rearrangement in lung adenocarcinoma harbouring epidermal growth factor receptor mutation: A single-center study
Zhong WX, Wei XF

Observational Study

- 12175** Prognostic values of optic nerve sheath diameter for comatose patients with acute stroke: An observational study

Zhu S, Cheng C, Wang LL, Zhao DJ, Zhao YL, Liu XZ

- 12184** Quality of care in patients with inflammatory bowel disease from a public health center in Brazil

Takamune DM, Cury GSA, Ferrás G, Herrerias GSP, Rivera A, Barros JR, Baima JP, Saad-Hossne R, Sasaki LY

- 12200** Comparison of the prevalence of sarcopenia in geriatric patients in Xining based on three different diagnostic criteria

Pan SQ, Li XF, Luo MQ, Li YM

Prospective Study

- 12208** Predictors of bowel damage in the long-term progression of Crohn's disease

Fernández-Clotet A, Panés J, Ricart E, Castro-Pocheiro J, Masamunt MC, Rodríguez S, Caballol B, Ordás I, Rimola J

Randomized Controlled Trial

- 12221** Protective effect of recombinant human brain natriuretic peptide against contrast-induced nephropathy in elderly acute myocardial infarction patients: A randomized controlled trial

Zhang YJ, Yin L, Li J

META-ANALYSIS

- 12230** Prognostic role of pretreatment serum ferritin concentration in lung cancer patients: A meta-analysis

Gao Y, Ge JT

CASE REPORT

- 12240** Non-surgical management of dens invaginatus type IIIB in maxillary lateral incisor with three root canals and 6-year follow-up: A case report and review of literature

Arora S, Gill GS, Saquib SA, Saluja P, Baba SM, Khateeb SU, Abdulla AM, Bavabeedu SS, Ali ABM, Elagib MFA

- 12247** Unusual presentation of Loeys-Dietz syndrome: A case report of clinical findings and treatment challenges

Azrad-Daniel S, Cupa-Galvan C, Farca-Soffer S, Perez-Zincer F, Lopez-Acosta ME

- 12257** Peroral endoscopic myotomy assisted with an elastic ring for achalasia with obvious submucosal fibrosis: A case report

Wang BH, Li RY

- 12261** Subclavian brachial plexus metastasis from breast cancer: A case report

Zeng Z, Lin N, Sun LT, Chen CX

- 12268** Case mistaken for leukemia after mRNA COVID-19 vaccine administration: A case report

Lee SB, Park CY, Park SG, Lee HJ

- 12278** Orthodontic-surgical treatment of an Angle Class II malocclusion patient with mandibular hypoplasia and missing maxillary first molars: A case report

Li GF, Zhang CX, Wen J, Huang ZW, Li H

- 12289** Multiple cranial nerve palsies with small angle exotropia following COVID-19 mRNA vaccination in an adolescent: A case report
Lee H, Byun JC, Kim WJ, Chang MC, Kim S
- 12295** Surgical and nutritional interventions for endometrial receptivity: A case report and review of literature
Hernández-Melchor D, Palafox-Gómez C, Madrazo I, Ortiz G, Padilla-Viveros A, López-Bayghen E
- 12305** Conversion therapy for advanced penile cancer with tislelizumab combined with chemotherapy: A case report and review of literature
Long XY, Zhang S, Tang LS, Li X, Liu JY
- 12313** Endoscopic magnetic compression stricturoplasty for congenital esophageal stenosis: A case report
Liu SQ, Lv Y, Luo RX
- 12319** Novel *hydroxymethylbilane synthase* gene mutation identified and confirmed in a woman with acute intermittent porphyria: A case report
Zhou YQ, Wang XQ, Jiang J, Huang SL, Dai ZJ, Kong QQ
- 12328** Modified fixation for periprosthetic supracondylar femur fractures: Two case reports and review of the literature
Li QW, Wu B, Chen B
- 12337** Erbium-doped yttrium aluminum garnet laser and advanced platelet-rich fibrin+ in periodontal diseases: Two case reports and review of the literature
Tan KS
- 12345** Segmental artery injury during transforaminal percutaneous endoscopic lumbar discectomy: Two case reports
Cho WJ, Kim KW, Park HY, Kim BH, Lee JS
- 12352** Pacemaker electrode rupture causes recurrent syncope: A case report
Zhu XY, Tang XH, Huang WY
- 12358** Hybrid intercalated duct lesion of the parotid: A case report
Stankevicius D, Petroska D, Zaleckas L, Kutanovaite O
- 12365** Clinical features and prognosis of multiple myeloma and orbital extramedullary disease: Seven cases report and review of literature
Hu WL, Song JY, Li X, Pei XJ, Zhang JJ, Shen M, Tang R, Pan ZY, Huang ZX
- 12375** Colon mucosal injury caused by water jet malfunction during a screening colonoscopy: A case report
Patel P, Chen CH
- 12380** Primary malignant pericardial mesothelioma with difficult antemortem diagnosis: A case report
Oka N, Orita Y, Oshita C, Nakayama H, Teragawa H
- 12388** Typical imaging manifestation of neuronal intranuclear inclusion disease in a man with unsteady gait: A case report
Gao X, Shao ZD, Zhu L

- 12395** Multimodality imaging and treatment of paranasal sinuses nuclear protein in testis carcinoma: A case report
Huang WP, Gao G, Qiu YK, Yang Q, Song LL, Chen Z, Gao JB, Kang L
- 12404** T1 rectal mucinous adenocarcinoma with bilateral enlarged lateral lymph nodes and unilateral metastasis: A case report
Liu XW, Zhou B, Wu XY, Yu WB, Zhu RF
- 12410** Influence of enhancing dynamic scapular recognition on shoulder disability, and pain in diabetics with frozen shoulder: A case report
Mohamed AA
- 12416** Acute myocardial necrosis caused by aconitine poisoning: A case report
Liao YP, Shen LH, Cai LH, Chen J, Shao HQ
- 12422** Danggui Sini decoction treatment of refractory allergic cutaneous vasculitis: A case report
Chen XY, Wu ZM, Wang R, Cao YH, Tao YL
- 12430** Phlegmonous gastritis after biloma drainage: A case report and review of the literature
Yang KC, Kuo HY, Kang JW
- 12440** Novel *TINF2* gene mutation in dyskeratosis congenita with extremely short telomeres: A case report
Picos-Cárdenas VJ, Beltrán-Ontiveros SA, Cruz-Ramos JA, Contreras-Gutiérrez JA, Arámbula-Meraz E, Angulo-Rojo C, Guadrón-Llanos AM, Leal-León EA, Cedano-Prieto DM, Meza-Espinoza JP
- 12447** Synchronous early gastric and intestinal mucosa-associated lymphoid tissue lymphoma in a *Helicobacter pylori*-negative patient: A case report
Lu SN, Huang C, Li LL, Di LJ, Yao J, Tuo BG, Xie R

LETTER TO THE EDITOR

- 12455** Diagnostic value of metagenomics next-generation sequencing technology in disseminated strongyloidiasis
Song P, Li X
- 12458** Diagnostic value of imaging examination in autoimmune pancreatitis
Wang F, Peng Y, Xiao B

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Cases*, Cornelia Bala, MD, PhD, Professor, Department of Diabetes and Nutrition Diseases, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca 400006, Romania. cbala@umfcluj.ro

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Cases* (WJCC, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

INDEXING/ABSTRACTING

The WJCC is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, Current Contents®/Clinical Medicine, PubMed, PubMed Central, Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 Edition of Journal Citation Reports® cites the 2021 impact factor (IF) for WJCC as 1.534; IF without journal self cites: 1.491; 5-year IF: 1.599; Journal Citation Indicator: 0.28; Ranking: 135 among 172 journals in medicine, general and internal; and Quartile category: Q4. The WJCC's CiteScore for 2021 is 1.2 and Scopus CiteScore rank 2021: General Medicine is 443/826.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Ying-Yi Yuan*; Production Department Director: *Xiang Li*; Editorial Office Director: *Jin-Lei Wang*.

NAME OF JOURNAL

World Journal of Clinical Cases

ISSN

ISSN 2307-8960 (online)

LAUNCH DATE

April 16, 2013

FREQUENCY

Thrice Monthly

EDITORS-IN-CHIEF

Bao-Gan Peng, Jerzy Tadeusz Chudek, George Kontogeorgos, Maurizio Serati, Ja Hyeon Ku

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2307-8960/editorialboard.htm>

PUBLICATION DATE

November 26, 2022

COPYRIGHT

© 2022 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>



Novel *hydroxymethylbilane synthase* gene mutation identified and confirmed in a woman with acute intermittent porphyria: A case report

Yu-Qing Zhou, Xiao-Qing Wang, Jun Jiang, Shu-Ling Huang, Zhuo-Jin Dai, Qiao-Qiong Kong

Specialty type: Medicine, research and experimental

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ata F, Qatar;
Giacomelli L, Italy

Received: July 11, 2022

Peer-review started: July 11, 2022

First decision: September 25, 2022

Revised: October 10, 2022

Accepted: November 2, 2022

Article in press: November 2, 2022

Published online: November 26, 2022



Yu-Qing Zhou, Xiao-Qing Wang, Shu-Ling Huang, Department of Endocrinology, Dongguan Hospital of Traditional Chinese Medicine, Dongguan 523003, Guangdong Province, China

Jun Jiang, Department of Science and Technology Services China Beijing Macro and Micro Test Biotech Co. Ltd, Beijing 100318, China

Zhuo-Jin Dai, The First Clinical Medical College, Guangdong Medical University, Zhanjiang 523003, Guangdong Province, China

Qiao-Qiong Kong, Department of Medicine, Wanjiang People's Hospital of Dongguan, Dongguan 523003, Guangdong Province, China

Corresponding author: Shu-Ling Huang, BSc, Professor, Department of Endocrinology, Dongguan Hospital of Traditional Chinese Medicine, No. 22 Songshanhu Avenue, Dongguan 523003, Guangdong Province, China. huangshuling_email@qq.com

Abstract

BACKGROUND

Acute intermittent porphyria (AIP) is a rare autosomal dominant porphyrin metabolic disease caused by a mutation in the *hydroxymethylbilane synthase* (HMBS) gene. This study aimed to explore the clinical manifestations of a patient with AIP, to identify a novel HMBS gene mutation in the proband and some of her family members, and to confirm the pathogenicity of the variant.

CASE SUMMARY

A 22-year-old Chinese woman developed severe abdominal pain, lumbago, sinus tachycardia, epileptic seizure, hypertension, and weakness in lower limbs in March, 2018. Biochemical examinations indicated hypohepatia and hyponatremia. Her last menstrual period was 45 d prior to admission, and she was unaware of the pregnancy, which was confirmed by a pregnancy test after admission. Sunlight exposure of her urine sample for 1 h turned it from yellow to wine red. Urinary porphyrin test result was positive. Based on these clinical manifestations, AIP was diagnosed. After increasing her daily glucose intake (250–300 g/d), abdominal pain was partially relieved. Three days after hospitalization, spontaneous vaginal bleeding occurred, which was confirmed as spontaneous abortion; thereafter, her clinical symptoms completely resolved. Genetic testing revealed a novel heterozygous splicing variant of the HMBS gene in exon 10 (c.648_651+1del-

CCAGG) in the proband and four other family members. The pathogenicity of the variant was verified through bioinformatic methods and a minigene assay.

CONCLUSION

We identified a novel *HMBS* gene mutation in a Chinese patient with AIP and confirmed its pathogenicity.

Key Words: Acute intermittent porphyria; *Hydroxymethylbilane synthase* gene; Novel mutation; Minigene assay; Bioinformatics analysis; Case report

©The Author(s) 2022. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The possible pathogenic loci in a woman with acute intermittent porphyria were identified by direct sequencing, which revealed a novel heterozygous splicing mutation (c.648_651+1delCCAGG) in exon 10 of *hydroxymethylbilane synthase* gene. Aberrant splicing, which led to the production of a truncated protein, was confirmed through an *in vitro* minigene assay and bioinformatics analysis.

Citation: Zhou YQ, Wang XQ, Jiang J, Huang SL, Dai ZJ, Kong QQ. Novel *hydroxymethylbilane synthase* gene mutation identified and confirmed in a woman with acute intermittent porphyria: A case report. *World J Clin Cases* 2022; 10(33): 12319-12327

URL: <https://www.wjgnet.com/2307-8960/full/v10/i33/12319.htm>

DOI: <https://dx.doi.org/10.12998/wjcc.v10.i33.12319>

INTRODUCTION

Acute intermittent porphyria (AIP) is a rare metabolic disease, and its incidence is difficult to accurately calculate. Reports vary between different countries and regions, the incidence of symptomatic AIP is 5.9 *per* million in Europe, 6.3 *per* million in Spain, 7.6 in France and 10-15 in Switzerland[1]. Available reports have demonstrated that AIP has various clinical manifestations. Typical clinical features during an acute attack of AIP, also known as overt AIP, include abdominal pain, constipation, nausea, vomiting, tachycardia, hypertension, muscle weakness, respiratory failure, epilepsy, and hyponatremia. Cases with posterior reversible encephalopathy syndrome have also been reported recently[1-3]. AIP attacks can be stimulated through several factors including sex hormone changes (estrogen and progesterone) involved in menstruation, pregnancy and childbirth, starvation, emotional fluctuations, excessive fatigue, and alcohol[4,5]. Drugs and bacterial infections also play an important role in the growth and development of various diseases including porphyria, which has been confirmed in recently published articles.[6]. Patients with latent AIP present with no or only mild clinical symptoms, and their biochemical tests may be normal[4,5]. Diagnosis of AIP is mainly dependent on clinical symptoms, biochemical examinations, and molecular analyses[7]. The detection of previously identified heterozygous *hydroxymethylbilane synthase* (*HMBS*) gene mutations through DNA analysis is the gold standard for diagnosis[7-11]. We report a case of a 22-year-old female diagnosed with AIP who was found to have a novel heterozygous mutation (c.648_651+1delCCAGG) in *HMBS* gene, which was also identified in her relatives. These findings could help the proband and related variant carriers to avoid life-threatening acute episodes. This case contributes to the existing knowledge on AIP and its causative genetic mutations.

CASE PRESENTATION

Chief complaints

A Chinese woman presented to our hospital because of severe abdominal pain and lumbago.

History of present illness

A 22-year-old Chinese woman developed severe abdominal pain and lumbago without obvious inducement 1 wk ago, the symptoms worsened accompanied by epileptic seizure and weakness in lower limbs for 3 d.

Table 1 Medical research council scale for muscle strength

Function of the muscle	Grade
No movement is observed	0
Only a trace or flicker of movement is seen or felt in the muscle or fasciculations are observed in the muscle	1
Muscle can move only if the resistance of gravity is removed	2
Muscle strength is further reduced such that the joint can be moved only against gravity with the examiner's resistance completely removed	3
Muscle strength is reduced but muscle contraction can still move joint against resistance	4
Muscle contracts normally against full resistance	5

History of past illness

In February, 2017, she gave birth to a baby after an unremarkable pregnancy. In July, 2017, she began to experience abdominal pain, back pain, and lumbago 5–10 d before her menstrual period. The pain was not severe and was relieved during menstruation.

Personal and family history

Neither the patient nor her family members had any remarkable medical history.

Physical examination

Her blood pressure was 160/100 mmHg and her heart rate was 110 bpm. Abdominal examination was normal with no tenderness or rebound pain elicited. Muscle tone in the extremities was normal, the muscle strength of both upper limbs was at level 5, and muscle strength in both lower limbs was at level 3 (Medical Research Council Scale for Muscle Strength is described in [Table 1](#)).

Laboratory examinations

Routine test results, such as kidney function, myocardial enzymes, and thyroid function, were normal. Serum chemistry findings were as follows: Hyponatremia (Na: 119–128 mmol/L; normal: 135–146 mmol/L), and hypohepatia (alanine aminotransferase: 272 IU/L, normal: 7–40 IU/L, aspartate aminotransferase: 72 IU/L, normal: 13–35 IU/L). When the patient's urine sample was exposed to sunlight for 1 h, it turned from yellow to wine red ([Figure 1](#)). A qualitative test of urinary porphyrin was positive.

Imaging examinations

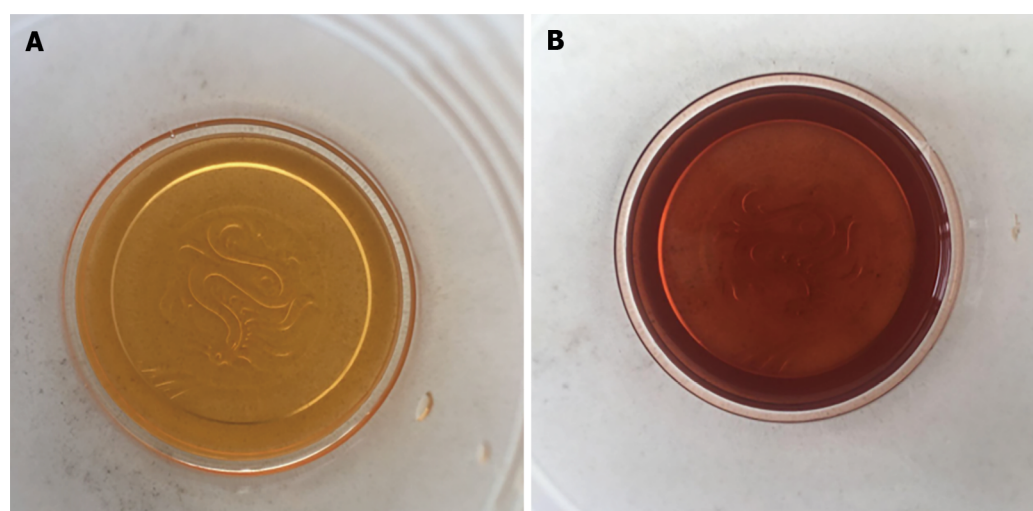
No radiological investigations were performed because she was pregnant and was diagnosed with AIP soon after admission.

Sanger sequencing

The study was approved by the ethics committee of Dongguan Hospital of Traditional Chinese Medicine [Ethical number: (2022), No. 15]. Genomic DNA was extracted from peripheral blood samples using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) and sent to the Macro-micro-test Company (Beijing, China). The *HMBS* (NM_000190) gene was analyzed by direct sequencing using a 3730XL DNA Analyzer (Applied Biosystems, Foster City, California). The sequence exhibited a heterozygous mutation (c.648_651+1delCCAGG) in exon 10 of *HMBS* gene of the proband ([Figure 2A](#)). In addition to the proband, four other family members were identified as carriers of the mutation: Her father (II.4), paternal uncle (II.1), younger brother (III.3), and son (IV.1) ([Figure 2B](#)). Her mother and two aunts tested negative for the mutant allele. All family members denied consanguinity, and no abnormalities were identified on physical or laboratory examinations.

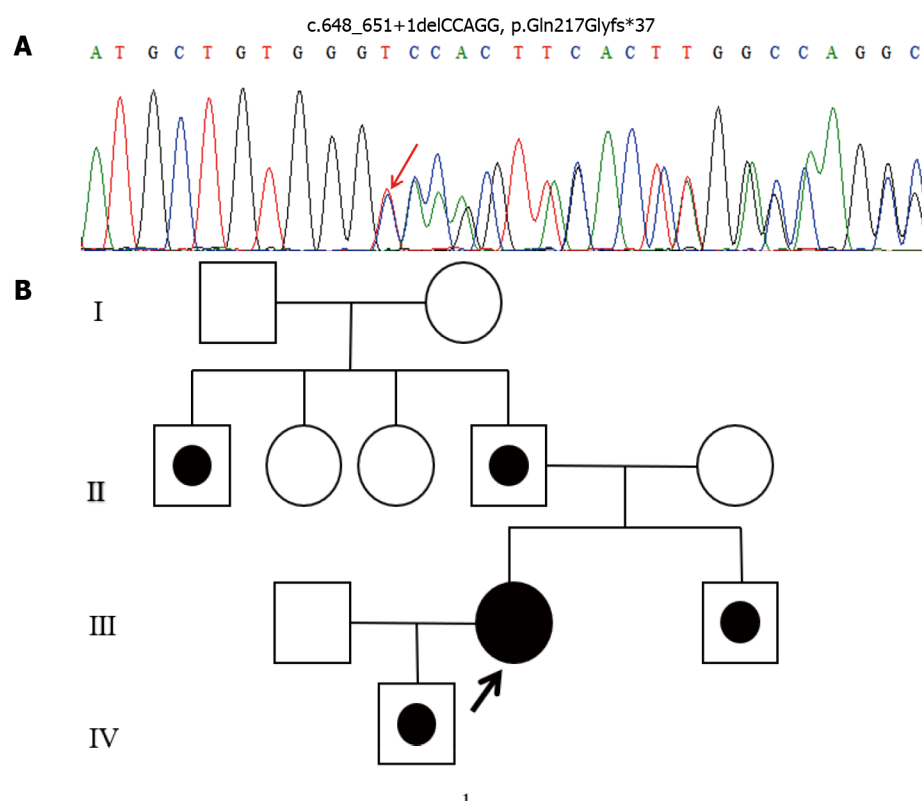
Minigene assay

To verify the functional effect of the c.648_651+1delCCAGG mutation in *HMBS* gene on pre-mRNA splicing, a minigene assay was carried out to evaluate the effect of the mutation on mRNA splicing ([Figure 3](#)). Wild-type and mutant minigenes were inserted into a pcMINI-C vector. The construction strategy inserted part of intron 9 (251 bp), exon 10 (39 bp), intron 10 (240 bp), and exon 11 (120 bp) of *HMBS* gene, in addition to exon A-intron A of MSC [exon A-intron A-intron 9 (251 bp)-exon 10 (39 bp)-intron 10 (240 bp)-and exon 11 (120 bp)] ([Figure 3A](#) and [C](#)). A total of four recombinant vectors were transfected into HeLa and 293T cells. After 48 h, four samples were collected, total RNA was extracted and reverse-transcribed into cDNA, and the cDNA was amplified using reverse transcription primers. Polymerase Chain Reaction (PCR) products were used to observe whether the exon A-exon 10-exon 11 transcript had a splicing pathogenic variant. Recombinant vectors showed that both wild-type and mutant minigenes were successfully inserted into the corresponding vectors ([Figure 3A](#)). Reverse



DOI: 10.12998/wjcc.v10.i33.12319 Copyright ©The Author(s) 2022.

Figure 1 The color of the patient's urine sample changed during an acute attack. Sunlight exposure of her urine sample for 1 h turned it from yellow to wine red. A: Yellow; B: Wine red.



DOI: 10.12998/wjcc.v10.i33.12319 Copyright ©The Author(s) 2022.

Figure 2 Gene sequencing and pedigree of the family. A: DNA sequencing showing the mutation c.648_651+1delCCAGG in *hydroxymethylbilane synthase* gene in the family members; B: Segregation of mutant alleles of the index patient (III.2). Solid symbols represent the overt proband indicated by an arrow, symbols with a dot in the middle represent asymptomatic latent family members carrying the mutation.

Transcription PCR for the PcMINI-C-HMBS-wt/mut minigene showed that, in both cell lines, the wild-type minigene had a band of the expected size (553 bp; Band a), while the mutant minigene had a larger band (Band b). When both were sequenced, Band a was a normally spliced band with the same splicing pattern as exon A-exon 10 (39 bp)-exon 11 (120 bp), while Band b retained the complete intron 10 sequence. Its splice pattern was exon A-exon 10 (39 bp)-▽intron 10 (239 bp)-exon 11 (120 bp) (Figure 3B and D). The mutation led to an overall retention of intron 10, turning this into a coding region. The minigene expression *in vitro* confirmed that the variant c.648_651+1delCCAGG could lead to aberrant mRNA splicing.

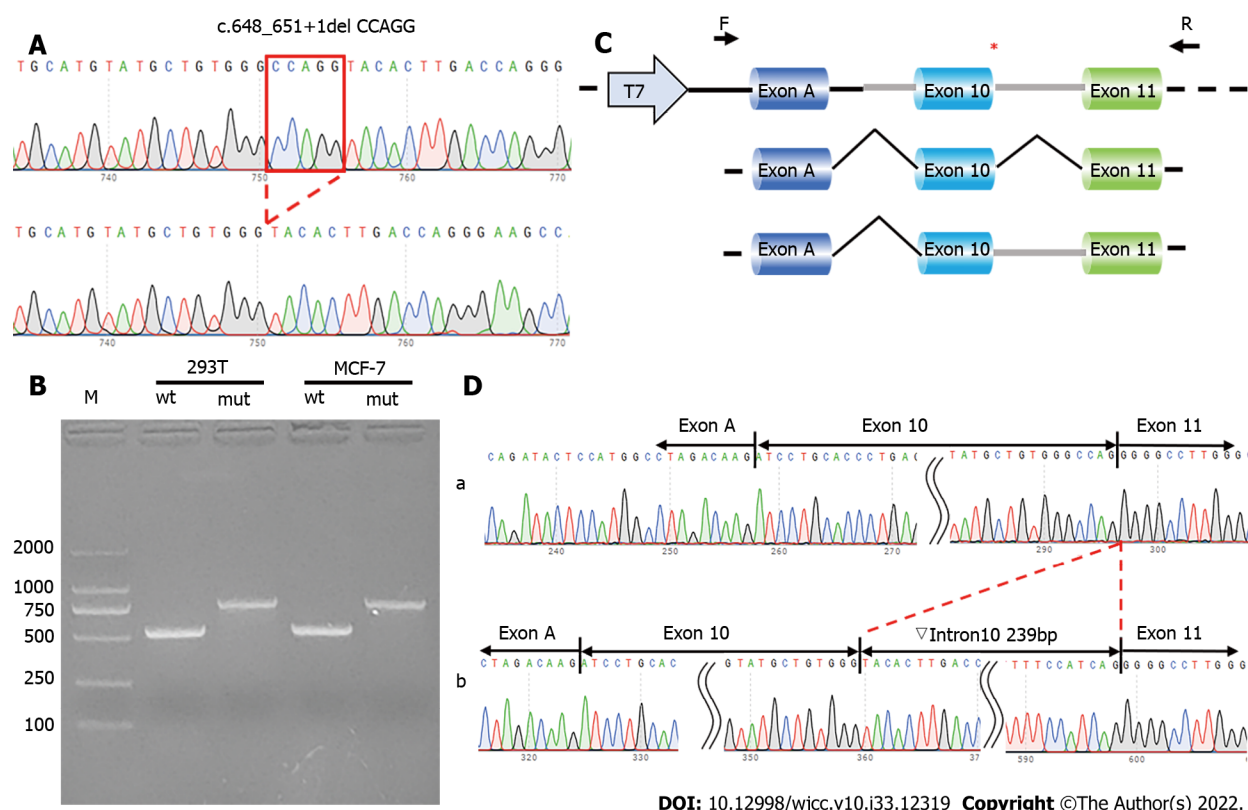


Figure 3 Validation of splicing alteration. A: Sequencing analysis minigenes: Top, mutant minigene containing c.648_651+1delCCAGG; bottom, wild-type minigene; B: Reverse Transcription Polymerase Chain Reaction of wild-type or mutant minigenes on agarose gel electrophoresis; C: Minigene construction and splicing pattern; D: Minigene splicing assay: Top, wild-type minigene; bottom, mutant minigene with retention of intron 10.

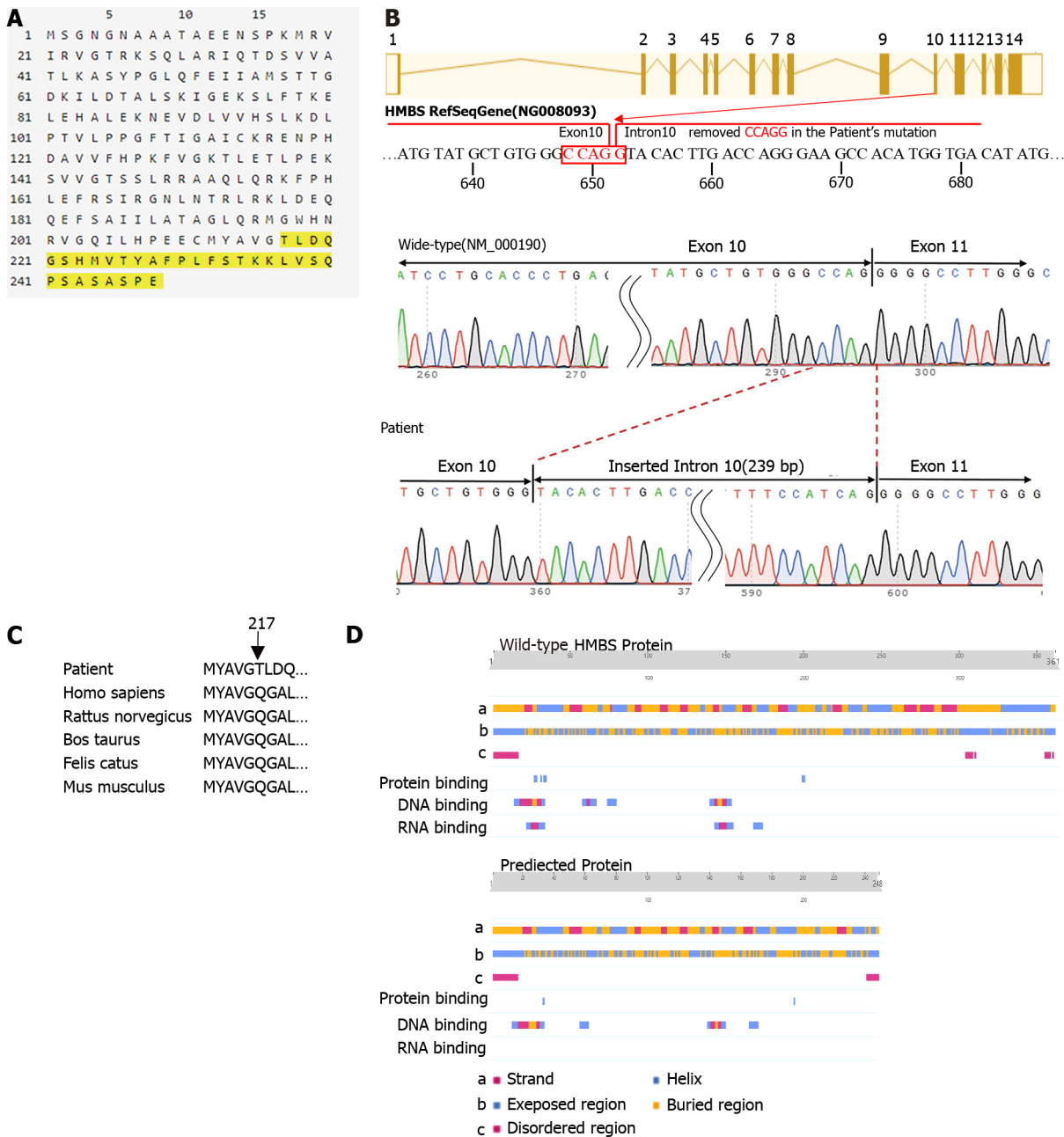
Bioinformatics analysis

The genotype of the proband and her family members was identified according to the sequencing results yielded by SeqMan software (Lasergene Genomics, DNASTAR, Inc., Madison, Wisconsin). The mutation identified in these individuals was not in the Human Gene Mutation Database (HGMD, <http://www.hgmd.org>), Exome Aggregation Consortium (<http://exac.broadinstitute.org>), or the 1000 Genomes Project (<http://www.1000genomes.org>). We used ORFfinder (<https://www.ncbi.nlm.nih.gov/orffinder>) to predict the reading frame of the amino acid sequence and PredictProtein (<https://www.predictprotein.org>) to analyze whether the secondary structure and function of proteins were altered by the mutation. ORFfinder demonstrated that the mutation site affects the normal translation of the protein. The c.648_651+1delCCAGG mutation caused the original donor recognition site of exon 10 to be destroyed. When the original donor recognition site was destroyed, the original splicing site might not have been spliced, resulting in retention of intron 10. Because the stranded intron 10 contains a stop codon, mRNA is terminated prematurely during translation, resulting in a protein variant with a length of 248 amino acids (Figure 4A and B). In comparison with the amino acid sequence of wild-type *HMBS*, the Q217T mutation of the new mutant occurred at site 217, and the translation was terminated after synthesis of 31 amino acids at the mutant site (Figure 4A and C). These findings are consistent with the results of the minigene assay.

We conducted further analysis using PredictProtein and found that the secondary structure of the protein also changed as follows: (1) The partially folded and helical regions of the truncated protein sequence were changed; (2) The exposed area of the surface was partially changed; (3) The disordered area at position 350 was missing; and (4) The RNA and protein binding regions had changed (Figure 4D).

FINAL DIAGNOSIS

The patient was ultimately diagnosed with AIP.



DOI: 10.12998/wjcc.v10.i33.12319 Copyright ©The Author(s) 2022.

Figure 4 Bioinformatics analysis. A: ORFfinder software predicted that the novel mutation gene translates a truncated protein (248 amino acids) with the deletion of 113 amino acids; B: The novel mutation in the *hydroxymethylbilane synthase* (HMBS) gene. The mutation shows that 5 bps (CCAGG) located at the intron and exon junction have been removed, resulting in the deletion of 4 bps of exon 10 and insertion of intron 10 (239 bps). The mutation site is represented by a red box; C: HMBS amino acid residues across different species; D: PredictProtein revealed an obvious difference between wild-type and predicted proteins in the strand and helix regions, exposed and buried regions, disordered regions, protein binding regions, and DNA and RNA binding region.

TREATMENT

The administration of high glucose (250–300 g/d) partially relieved the patient’s abdominal pain. Three days after hospitalization, the patient spontaneously aborted. This was followed by complete resolution of symptoms.

OUTCOME AND FOLLOW-UP

We advised the proband and the variant carriers to avoid triggers of acute attacks. In September, 2018, the patient had an unplanned pregnancy. Symptoms, such as abdominal pain and weakness, recurred. Considering her illness and at her request, we performed an abortion on the patient. All her symptoms

resolved after the termination of the pregnancy. She was advised to increase sugar intake and to use contraception. She was asymptomatic on follow-up. A timeline of her three pregnancies is shown in Figure 5.

DISCUSSION

AIP is a rare autosomal dominant metabolic disease caused by mutations in the gene that encodes *HMBS*[7,8,12]. The *HMBS* gene is located on chromosome 11q23.3 and includes 15 exons and two different promoters. To date, 504 mutations have been identified, of which 203 are point mutations, 107 are deletions mutations, 102 are splicing mutations, 48 are insertion mutations, and 44 are other types of mutations (HGMD, <http://www.hgmd.cf.ac.uk/ac/index.php>)[13]. A study from France has shown that the prevalence of symptomatic AIP is approximately 7.6 *per* million inhabitants, while pathogenetic mutations in the *HMBS* gene are observed in 1/1782[1,14]. This means that the penetrance of AIP is very low, being observed in only approximately 1% of the general population and 20%-50% of families with AIP[1,14]. In principle, AIP is an autosomal dominant metabolic disease, with no sex predilection[15]. However, female mutation carriers are more likely to experience acute attacks after puberty, which are attributed to shifting levels of estrogen and progesterone related to the menstrual cycle, while male mutation carriers occasionally have mild or no clinical symptoms[16]. In our study, the female variant carrier (proband) suffered pregnancy-induced acute attacks, which could be diagnosed based on clinical symptoms and biochemical examinations. Of the male variant carriers, two experienced irregular abdominal pain and constipation (her father and uncle), while two others (her younger brother and son) experienced no clinical symptoms. The observed penetrance of the disease and the different clinical manifestations between men and women were consistent with that of previous clinical reports[1,4-6].

Increased estrogen and progesterone during pregnancy can precipitate acute attacks of porphyria, and the pregnancy outcomes of women with AIP can include uneventful pregnancy, normal delivery, spontaneous abortion and termination of the fetus as a result of not only the pregnancy but also the high incidence of associated hyperemesis gravidarum and drug use[5,7]. This partly explains why manifestation of the mutation did not interfere in the patient's first pregnancy.

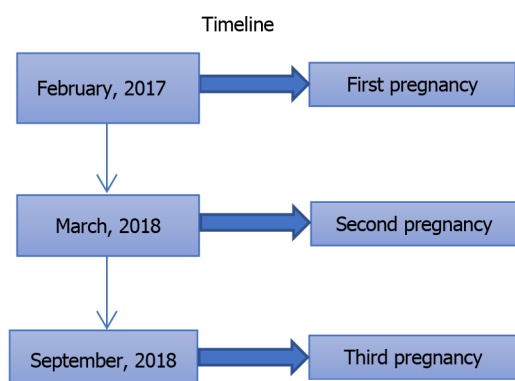
A novel splicing pathogenic variant (c.648_651+1delCCAGG) was identified in the family using Sanger sequencing. This mutation occurs in the junction region of exon 10 and intron 10, thus depriving the junction region of the GU-AG splice site[17] and preventing the intron cleavage enzyme from recognizing the splicing site; therefore, the whole of intron 10 is retained in the mature mRNA when the pre-mRNA is spliced. However, because the retained intron sequence contains a stop codon, it causes early termination of translation, potentially producing a shortened protein. ORFfinder predicted that the shortened protein sequence contains 248 amino acids. Compared with the wild-type protein sequence of 361 amino acids, the truncated protein sequence shows changes in the structure and function of the enzyme due to the deletion of 113 amino acids. The *in vitro* minigene expression also confirmed that the variant could lead to retention of intron 10 and aberrant mRNA splicing.

We predicted and analyzed the secondary structure of the truncated protein using PredictProtein and found that the beta-fold and alpha-helix sites in the shortened protein changed; furthermore, the DNA, RNA, and protein binding sites were altered. In particular, the lack of RNA binding sites may affect its function, because the interaction between protein and RNA plays an important role in many biological activities, such as post-transcriptional gene regulation and regulation of gene expression[18-20]. In addition, the mutant generated a new disordered region at position 247 but missed the disordered regions at positions 310 and 361 of the wild-type. Such disordered regions of proteins are also involved in gene signal transduction and regulation[21,22]. Therefore, the new mutant may cause disease due to loss of a functional region.

Fifty percent of missense mutations identified at the DNA and RNA levels have been confirmed to disrupt mRNA splicing[23]. So far, more than 500 *HMBS* gene variants that cause AIP have been verified in HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>), of which 108 led to splicing. We analyzed the pathogenicity of the c.648_651+1delCCAGG mutation using a bioinformatics method and *in vitro* minigene assay. To further explore the molecular pathogenesis of the mutation, we intend to detect the influence of the mutation on enzyme activity. We aim to conduct further research to determine whether the mutation can lead to nonsense-mediated mRNA decay as well as detect the presence of truncated proteins, degradation of truncated proteins, and interaction of downstream proteins *in vivo*. Furthermore, we intend to demonstrate the pathogenicity of gene mutation from multiple perspectives.

CONCLUSION

We identified a novel splicing mutation (c.648_651+1delCCAGG) in the *HMBS* gene of a Chinese woman with AIP and verified the pathogenicity of the variant through bioinformatics methods and an *in vitro* minigene assay. We provided the mutation carriers a clearer picture of their condition and



DOI: 10.12998/wjcc.v10.i33.12319 Copyright ©The Author(s) 2022.

Figure 5 A timeline of her three pregnancies. She had her first pregnancy and gave birth to a boy in February, 2017; she had a second pregnancy in March, 2018, but had a spontaneous abortion due to an acute attack of acute intermittent porphyria (AIP); she became pregnant for the third time in September, 2018, but had an abortion because of the acute attack of AIP.

provided recommendations on how to prevent potentially life-threatening acute episodes. Our discovery has also expanded the known spectrum of pathogenic *HMBS* gene mutations.

ACKNOWLEDGEMENTS

The authors thank the family members for providing blood samples, cooperating with treatment, and agreeing to participate in this study.

FOOTNOTES

Author contributions: Zhou YQ, Wang XQ and Jiang J drafted and finalized the manuscript; Dai ZJ and Kong QQ revised the manuscript; Huang SL designed and supervised the study; all authors read and approved the final manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <https://creativecommons.org/licenses/by-nc/4.0/>

Country/Territory of origin: China

ORCID number: Yu-Qing Zhou 0000000220116372; Xiao-Qing Wang 0000-0002-6362-983x; Jun Jiang 0000000271192682; Shu-Ling Huang 0000-0002-6082-0014; Zhuo-Jin Dai 0000-0003-1047-2549; Qiao-Qiong Kong 0000-0002-9153-9444.

S-Editor: Wei ZH

L-Editor: A

P-Editor: Wei ZH

REFERENCES

- 1 Ma L, Tian Y, Peng C, Zhang Y, Zhang S. Recent advances in the epidemiology and genetics of acute intermittent porphyria. *Intractable Rare Dis Res* 2020; **9**: 196-204 [PMID: 33139978 DOI: 10.5582/irdr.2020.03082]
- 2 Wylie K, Testai FD. Neurological Manifestations of Acute Porphyrins. *Curr Neurol Neurosci Rep* 2022; **22**: 355-362

- [PMID: 35665475 DOI: 10.1007/s11910-022-01205-7]
- 3 **Simon NG**, Herkes GK. The neurologic manifestations of the acute porphyrias. *J Clin Neurosci* 2011; **18**: 1147-1153 [PMID: 21724399 DOI: 10.1016/j.jocn.2011.01.003]
 - 4 **Marsden JT**, Rees DC. A retrospective analysis of outcome of pregnancy in patients with acute porphyria. *J Inherit Metab Dis* 2010; **33**: 591-596 [PMID: 20567908 DOI: 10.1007/s10545-010-9142-2]
 - 5 **Wang B**, Rudnick S, Cengia B, Bonkovsky HL. Acute Hepatic Porphyrins: Review and Recent Progress. *Hepatol Commun* 2019; **3**: 193-206 [PMID: 30766957 DOI: 10.1002/hep4.1297]
 - 6 **Ma Y**, Teng Q, Zhang Y, Zhang S. Acute intermittent porphyria: focus on possible mechanisms of acute and chronic manifestations. *Intractable Rare Dis Res* 2020; **9**: 187-195 [PMID: 33139977 DOI: 10.5582/irdr.2020.03054]
 - 7 **Fu Y**, Jia J, Yue L, Yang R, Guo Y, Ni X, Shi T. Systematically Analyzing the Pathogenic Variations for Acute Intermittent Porphyrin. *Front Pharmacol* 2019; **10**: 1018 [PMID: 31572191 DOI: 10.3389/fphar.2019.01018]
 - 8 **Hu Y**, Li W, Kang N, Ma L, Teng Q, Mo G, Wu J, Wang X, Bi R, Zhang S. Identification and molecular analysis of 17 novel variants of hydroxymethylbilane synthase in Chinese patients with acute intermittent porphyria. *Clin Genet* 2022; **101**: 116-121 [PMID: 34523126 DOI: 10.1111/cge.14063]
 - 9 **Morán-Jiménez MJ**, Borrero-Corte MJ, Jara-Rubio F, García-Pastor I, Díaz-Díaz S, Castelbón-Fernández FJ, Enríquez-de-Salamanca R, Méndez M. Molecular Analysis of 55 Spanish Patients with Acute Intermittent Porphyrin. *Genes (Basel)* 2020; **11** [PMID: 32806544 DOI: 10.3390/genes11080924]
 - 10 **Loskove Y**, Yasuda M, Chen B, Nazarenko I, Cody N, Desnick RJ. Acute hepatic porphyrias: Identification of 46 hydroxymethylbilane synthase, 11 coproporphyrinogen oxidase, and 20 protoporphyrinogen oxidase novel mutations. *Mol Genet Metab* 2019; **128**: 352-357 [PMID: 30385147 DOI: 10.1016/j.ymgme.2018.10.008]
 - 11 **Ren Y**, Xu LX, Liu YF, Xiang CY, Gao F, Wang Y, Bai T, Yin JH, Zhao YL, Yang J. A novel 55-basepair deletion of hydroxymethylbilane synthase gene found in a Chinese patient with acute intermittent porphyria and her family: A case report. *Medicine (Baltimore)* 2018; **97**: e12295 [PMID: 30212967 DOI: 10.1097/MD.00000000000012295]
 - 12 **Llewellyn DH**, Scobie GA, Urquhart AJ, Whatley SD, Roberts AG, Harrison PR, Elder GH. Acute intermittent porphyria caused by defective splicing of porphobilinogen deaminase RNA: a synonymous codon mutation at -22 bp from the 5' splice site causes skipping of exon 3. *J Med Genet* 1996; **33**: 437-438 [PMID: 8733062 DOI: 10.1136/jmg.33.5.437]
 - 13 **Yasuda M**, Chen B, Desnick RJ. Recent advances on porphyria genetics: Inheritance, penetrance & molecular heterogeneity, including new modifying/causative genes. *Mol Genet Metab* 2019; **128**: 320-331 [PMID: 30594473 DOI: 10.1016/j.ymgme.2018.11.012]
 - 14 **Chen B**, Solis-Villa C, Hakenberg J, Qiao W, Srinivasan RR, Yasuda M, Balwani M, Doheny D, Peter I, Chen R, Desnick RJ. Acute Intermittent Porphyrin: Predicted Pathogenicity of HMBS Variants Indicates Extremely Low Penetrance of the Autosomal Dominant Disease. *Hum Mutat* 2016; **37**: 1215-1222 [PMID: 27539938 DOI: 10.1002/humu.23067]
 - 15 **Kauppinen R**, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. *Clin Chem* 2002; **48**: 1891-1900 [PMID: 12406973 DOI: 10.1093/clinchem/48.11.1891]
 - 16 **Vassiliou D**, Lempessi C, Harper P, Sardh E. Challenges in the management of acute intermittent porphyria with recurrent attacks during pregnancy: A case report. *Clin Case Rep* 2020; **8**: 2483-2487 [PMID: 33363763 DOI: 10.1002/ccr3.3185]
 - 17 **Maeda N**, Horie Y, Sasaki Y, Ueta E, Adachi K, Nanba E, Kawasaki H, Kudo Y, Kondo M. A splicing mutation in the hydroxymethylbilane synthase gene in a Japanese family with acute intermittent porphyria. *Clin Biochem* 1999; **32**: 411-417 [PMID: 10667475 DOI: 10.1016/S0009-9120(99)00043-0]
 - 18 **Nechay M**, Kleiner RE. High-throughput approaches to profile RNA-protein interactions. *Curr Opin Chem Biol* 2020; **54**: 37-44 [PMID: 31812895 DOI: 10.1016/j.cbpa.2019.11.002]
 - 19 **Corley M**, Burns MC, Yeo GW. How RNA-Binding Proteins Interact with RNA: Molecules and Mechanisms. *Mol Cell* 2020; **78**: 9-29 [PMID: 32243832 DOI: 10.1016/j.molcel.2020.03.011]
 - 20 **Graindorge A**, Pinheiro I, Nawrocka A, Mallory AC, Tsvetkov P, Gil N, Carolis C, Buchholz F, Ulitsky I, Heard E, Taipale M, Shkumatava A. In-cell identification and measurement of RNA-protein interactions. *Nat Commun* 2019; **10**: 5317 [PMID: 31757954 DOI: 10.1038/s41467-019-13235-w]
 - 21 **Uversky VN**. Functional roles of transiently and intrinsically disordered regions within proteins. *FEBS J* 2015; **282**: 1182-1189 [PMID: 25631540 DOI: 10.1111/febs.13202]
 - 22 **Wright PE**, Dyson HJ. Intrinsically disordered proteins in cellular signalling and regulation. *Nat Rev Mol Cell Biol* 2015; **16**: 18-29 [PMID: 25531225 DOI: 10.1038/nrm3920]
 - 23 **López-Bigas N**, Audit B, Ouzounis C, Parra G, Guigó R. Are splicing mutations the most frequent cause of hereditary disease? *FEBS Lett* 2005; **579**: 1900-1903 [PMID: 15792793 DOI: 10.1016/j.febslet.2005.02.047]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

Telephone: +1-925-3991568

E-mail: bpgoffice@wjgnet.com

Help Desk: <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

