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Case report: Two cases of Hb Liuzhou-Yufeng (*HBA1*:c.334G>T) found in Northern Thailand

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ABSTRACT

Background: Hemoglobin (Hb) Liuzhou-Yufeng is an α-globin variant caused by a heterozygous *HBA1* mutation (c.334G>T). However, its clinical significance is not well-defined. Moreover, it is hard to detect using standard capillary electrophoresis (CE) or high-performance liquid chromatography (HPLC) methods and often need molecular techniques like next-generation sequencing (NGS).

Objective: This study aims to describe genotype and hematological features of the first two cases with heterozygosity for Hb Liuzhou-Yufeng found in Northern Thailand and the diagnostic challenges faced.

Materials and methods: Two patients with anemia, a 51-year-old female and a 32-year-old male, underwent hematological evaluation, Hb analysis using CE for case 1 and HPLC for case 2, iron studies, and red cell morphology tests. The dichlorophenolindophenol (DCIP) and osmotic fragility (OF) tests were also conducted. The molecular work-up included multiplex real-time PCR for α-thalassemia deletions, followed by NGS.

Results: Both patients were confirmed by the NGS to carry Hb Liuzhou-Yufeng ($\alpha\alpha^{LY}/\alpha\alpha$). Their total Hb levels were found within the ranges of mild anemia (10-12 g/dL). However, they had normocytic and normochromic red cells, and the morphology of the red blood cells appeared to be normal. In addition, the Hb analysis by HPLC and CE methods revealed normal peaks and levels of HbA, HbA_2, and HbF.

Conclusion: Hb Liuzhou-Yufeng seems clinically harmless but is challenging to detect using conventional methods. This study highlights the importance of NGS in finding rare Hb variants. It improves diagnostic accuracy, genetic counseling, and personalized management of hemoglobinopathy.

Introduction

Inherited hemoglobin (Hb) disorders rank among the most prevalent genetic conditions globally and are generally divided into thalassemia (deficiencies in globin chain synthesis) and structural hemoglobinopathies (defects caused by abnormal globin chains).[1,2] In Southeast Asia, α-thalassemia is notably common, with carrier rates reaching 30-40% in Thailand, particularly in the northern areas. Most cases result from α-globin gene deletions like the $--^{SEA}$ and $-\alpha^{3.7}$ deletions, but non-deletional variants such as Hb

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Constant Spring (HbCS, *HBA2*:c.427T>C) and Hb Paksé (HbPS, *HBA2*:429A>T) are also prevalent.[3–5] Although regular hematological assessments and Hb analysis by capillary electrophoresis (CE) or high-performance liquid chromatography (HPLC) detect common variants, they often miss rare mutations. Consequently, molecular techniques, such as next-generation sequencing (NGS), are necessary for precise diagnosis, particularly in unusual cases.[4]

A new α-globin variant, Hb Liuzhou, was initially reported in China and results from a point mutation in *HBA1* (c.182A>G), causing a lysine-to-arginine at codon 60. It was named according to the birthplace of the patient.[6] Carriers usually show no symptoms and have normal blood indices.[6,7] Later, a different yet associated variant, Hb Liuzhou-Yufeng (*HBA1*:c.334G>T; Ala→Ser), was discovered. The "Yufeng" is one of four districts in Liuzhou, a prefecture-level city within the Guangxi Zhuang Autonomous Region of China; hence, the Hb Liuzhou-Yufeng was named according to the geographic origin of the initial case. Although Hb Liuzhou is recognized as clinically silent, the clinical significance of Hb Liuzhou-Yufeng remains uncertain due to the scant literature available.[8] Thus, we present the initial two cases of Hb Liuzhou-Yufeng identified in Chiang Mai, Northern Thailand. These instances highlight the diagnostic difficulties presented by this uncommon variant and stress the significance of molecular testing in screening for thalassemia and hemoglobinopathy.

Case Presentation

Case 1: A 51-year-old female patient presented with vertigo persisting for four days, accompanied by generalized weakness. Complete blood count revealed Hb concentration of 11.4 gm/dL, mean corpuscular volume (MCV) of 86.0 fL, and mean corpuscular Hb (MCH) of 28.0 pg, indicating mild normocytic normochromic anemia. The morphology of the red blood cells appeared to be normal, and the findings from both the DCIP and OF tests were negative. Further Hb analysis through CE (Capillarys 2 Flex piercing, Sebia, Evry, France) was performed, yielding results within the normal range (Figure 1A and Table 1), thereby excluding a β-thalassemia trait. However, a potential α-thalassemia syndrome was still suspected. Subsequent to these findings, molecular testing was conducted using a single-tube multiplex real-time PCR with Evagreen and high-resolution melting (HRM) analysis to identify common α^0-thalassemia deletions, specifically $--^{SEA}$, $--^{THAI}$, and $--^{CR}$ deletions.[7] In addition, the common α^+-thalassemia $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions were investigated through a multiplex Gap-PCR technique.[8] The patient tested negative for these mutations. Following the negative PCR results, further genetic analysis employing NGS was performed. The NGS panel designed to target the coding regions of the *HBA1*, *HBA2*, and *HBB* genes. This assay utilized the Thalassemia Gene Detection Kit provided by BGI Group (Shenzhen, China). Subsequently, sequence reads were mapped against the human genome reference hg19, and detected genetic variants were analyzed using THACARE HALOS software (BGI Group). It revealed a mutation from GCC to TCC at codon 111 of the α1-globin gene, identified as Hb Liuzhou-Yufeng variant (Figure 2).

Case 2: A 32-year-old HIV infected male came in with weakness and nausea for 24 hours. His Hb level was 10.7 gm/dL, showing mild anemia. The MCV was 94.0 fL and MCH was 31.9 pg, indicating normocytic normochromic anemia. The red cell distribution width (RDW) was 16.9%. Red cell appearance was normal, and both DCIP and OF tests were negative. Iron studies indicated elevated ferritin levels, decreased serum iron and total iron-binding capacity (TIBC), and normal transferrin saturation (Table 1), so refusing iron deficiency anemia. The Hb analysis by HPLC (VARIANT β-thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA, USA) was normal (Figure 1B and Table 1), thereby excluding a β-thalassemia trait. However, a potential α-thalassemia syndrome was still suspected. Subsequent to these findings, molecular testing was conducted using a single-tube multiplex real-time PCR with Evagreen and HRM analysis to identify common α^0-thalassemia deletions, specifically $--^{SEA}$, $--^{THAI}$, and $--^{CR}$ deletions.[7] In addition, the common α^+-thalassemia $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions were investigated through a multiplex Gap-PCR technique.[8] The patient tested negative for these mutations. Later, the NGS revealed a mutation from GCC to TCC at codon 111 of the α1-globin gene, identified as Hb Liuzhou-Yufeng variant.

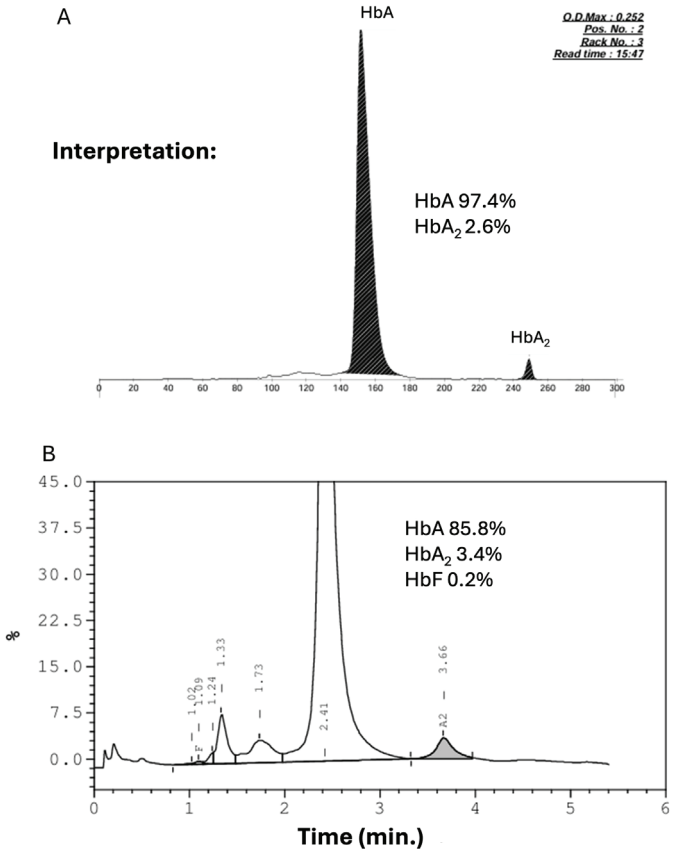

Figure 1. *CE electropherogram and HPLC chromatogram without seeing the separate peak of Hb Liuzhou-Yufeng. A: case 1, B: case 2.*

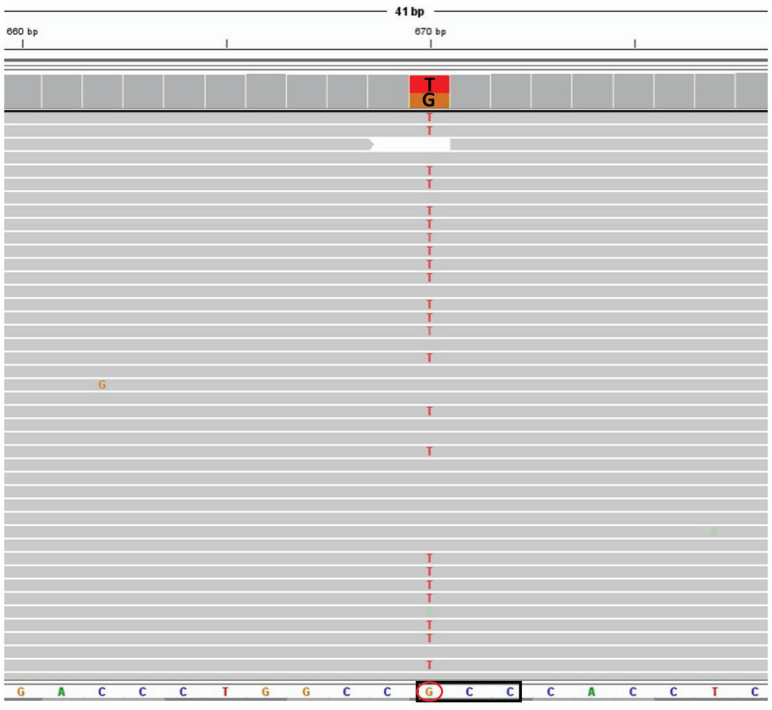

Figure 2. *Representative of NGS results exported from the integrative genomics viewer (IGV) of Hb Liuzhou-Yufeng (HBA1:c.334G>T).*

Table 1. The characteristics and hematological parameters of cases

Characteristics and hematological parameters	Case No. 1	Case No. 2
Age (Years)	51	32
Gender	Female	Male
α-globin genotype	$\alpha\alpha^{LY}/\alpha\alpha$	$\alpha\alpha^{LY}/\alpha\alpha$
β-globin genotype	β^A/β^A	β^A/β^A
RBCs ($x10^{12}$/L)	4.13	3.4
Total Hb (gm/dL)	11.4	10.7
PCV (L/L)	0.35	0.32
MCV (fL)	86.0	94.0
MCH (pg)	28.0	31.0
MCHC (gm/L)	325	334.4
RDW (%)	15.3	16.9%
Ferritin (ng/mL)	N/A	2,020
Serum iron (µg/dL)	N/A	45
TIBC (µg/dL)	N/A	175
Transferrin saturation (%)	N/A	25.7
HbA (%)	97.4†	85.8*
HbA_2 (%)	2.6†	3.4*
HbF (%)	0.0†	0.2*

Note: *Normal range of adults: red blood cell counts (RBCs) 4.2-6.1 x 10^{12}/L, total Hb 12.0-18.0 gm/dL, packed cell volume (PCV) 0.37-0.52 L/L, mean corpuscular volume (MCV) 80-100 fL, mean corpuscular Hb (MCH) 27.0-31.0 pg, mean corpuscular Hb concentration (MCHC) 320-360 gm/L, red cell distribution width (RDW) 11.5-15.0%, HbA 95.0-98.0%, HbA2 1.5-3.5%, HbF 0.0-1.0%, Ferritin 30-300 ng/mL, Serum iron 50-150 µg/dL, total iron-binding capacity (TIBC) 240-450 µg/dL, Transferrin saturation 20-55%. †Hb analysis by capillary electrophoresis (CE), *Hb analysis by high-performance liquid chromatography (HPLC)*

Discussion

The α-globin genes, *HBA1* and *HBA2*, are located on chromosome 16p13.3. They encode nearly identical α-globin chains. *HBA2* produces about three times more transcripts than *HBA1* in red blood cells. Changes in these genes cause α-thalassemia, with deletions being more common. Non-deletional mutations in *HBA2* typically lead to more severe symptoms. This is because disrupting the higher-expressing gene significantly reduces α-globin production. On the other hand, changes in *HBA1* usually result in milder effects due to its lower baseline expression. [9,10]

In both individuals in the current study, mild to moderate Hb reduction was noted along with normocytic normochromic anemia. The significantly elevated ferritin level (2,020 ng/mL) in Case 2 may be attributed to HIV infection, as HIV induces chronic inflammation, leading to an increase in ferritin levels. Hb analysis through CE (case 1) and HPLC (case 2) revealed no Hb variants. Molecular testing excluded significant α-thalassemia deletions ($--^{SEA}$, $--^{THAI}$, and $--^{CR}$). The NGS revealed an identical point mutation in the *HBA1* gene (c.334G>T), leading to the Hb Liuzhou-Yufeng variant. The substitution from alanine (nonpolar) to serine (polar) at codon 111 may subtly affect Hb structure and oxygen affinity, potentially accounting for mild symptoms.[11,12] While this mutation doesn't alter Hb levels identifiable through Hb typing and often results in relatively mild anemia, the subtle clinical symptoms noted may be attributed to the variant's influence on the oxygen affinity of Hb. Its placement in *HBA1*, a gene with reduced expression relative to *HBA2*, also contributes to the attenuated severity.

Research from Northern Thailand has shown that combined α-thalassemia mutations can produce a wide range of clinical and hematological phenotypes, often influenced by coexisting variants such as HbE or α-thalassemia deletions.[13] For example, the silent Hb

Hekinan II variant (*HBA1*:c.84G>T) remains clinically harmless unless co-inherited with other α-thalassemia mutations.[14] Currently, there are no published reports of combined mutations involving Hb Liuzhou-Yufeng. Because this variant locates in the less-expressed *HBA1* gene, its pathogenic effect is usually mild. However, when co-inherited with α-thalassemia deletions (e.g., α^{+}-thalassemia), it could present as a mild phenotype characterized by slight anemia or thalassemia features, reflecting the altered balance between α- and β-globin chain synthesis.

From a diagnostic perspective, these cases emphasize the limitations of Hb analysis methods like CE and HPLC, which showed normal outcomes even with the presence of the Hb Liuzhou-Yufeng variant. This underlines the risk of underdiagnosis if laboratories rely solely on routine Hb analysis. Diagnostic workflows should incorporate the NGS and other molecular tools, especially in areas with a high occurrence of thalassemia and hemoglobinopathies, to guarantee precise identification of rare variants. Therefore, it is recommended that NGS be performed on all anemic samples, even when red blood cells are normocytic and normochromic, and common causes of anemia remain undetected. Accurate diagnosis is crucial for effective genetic counseling, family risk evaluation, and guiding public health initiatives for thalassemia management.

Conclusion

This research demonstrated the effective application of a targeted NGS panel for the identification of rare α1-globin gene mutation, Hb Liuzhou-Yufeng. Clinically, individuals carrying Hb Liuzhou-Yufeng showed few or no symptoms, reflecting a benign clinical course, which highlights the difficulty of diagnosing this variant through clinical evaluation or conventional screening methods alone. Therefore, integrating advanced molecular techniques such as NGS into thalassemia screening programs is essential for the accurate identification of rare Hb variants. This approach not only enhances diagnostic precision but also supports improved genetic counseling and personalized clinical management, ultimately leading to better outcomes for individuals affected by hemoglobinopathies.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (AMSEC-68EM-014).

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

CRediT authorship contribution statement

Yasmina Azfa Putri Alif: conceptualization, investigation, writing original draft; **Maulida Syifa Kamila Makarim:** conceptualization, investigation, writing original draft; **Chedtapak Ruengdit:** blood and data collections, investigation, methodology, review and editing; **Yona Mimanda:** conceptualization, review and editing; **Chris Adhiyanto:** conceptualization, review and editing; **Laifa Annisa Hendarmin:** conceptualization, review and editing; **Sakorn Pornprasert:** conceptualization, project administration, validation, writing, reviewing and editing.

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References

[1] Leckngam P. Thalassemia and hemoglobinopathies in Thailand: A systematic review. J Health Sci Altern Med. 2023; 5(3): 104-13. doi: 10.14456/jhsam.2023.24.

[2] WHO. Regional desk review of haemoglobinopathies with an emphasis on thalassaemia and accessibility and availability of safe blood and blood products as per these patients' requirement in South-East Asia under universal health coverage [Internet]. 2021. Available from: http://apps.who.int/bookorders.

[3] Jomoui W, Panyasai S, Sripornsawan P, Tepakhan W. Revisiting and updating molecular epidemiology of α-thalassemia mutations in Thailand using MLPA and new multiplex gap-PCR for nine α-thalassemia deletion. Sci Rep. 2023; 13(1): 9850. doi: 10.1038/s41598-023-36840-8.

[4] Musallam KM, Cappellini MD, Coates TD, Kuo KHM, Al-Samkari H, Sheth S, *et al*. Alpha-thalassemia: A practical overview. Blood Rev. 2024; 64: 101165. doi: 10.1016/j.blre.2023.101165.

[5] Paiboonsukwong K, Jopang Y, Winichagoon P, Fucharoen S. Thalassemia in Thailand. Hemoglobin. 2022; 46(1): 53-7. doi:10.1080/03630269.2022.2025824.

[6] Xu A, Chen W, Xie W, Ji L. Identification of a new hemoglobin variant Hb Liuzhou [HBA1:C.182A→G] by MALDI-TOF mass spectrometry during HbA1c measurement. Scand J Clin Lab Invest. 2020; 80(6): 479-83. doi: 10.1080/00365513.2020.1783698.

[7] Ruengdit C, Punyamung M, Intasai N, Pornprasert S. Single-tube multiplex real-time PCR with EvaGreen and high-resolution melting analysis for diagnosis of α0-thalassemia--SEA,--THAI, and--CR type deletions. PLoS One. 2023; 18(11): e0293838. doi: 10.1371/journal.pone.0293838.

[8] Fucharoen S, Fucharoen G, Sanchaisuriya K, Pengjam Y. Molecular analysis of a Thai β-thalassaemia heterozygote with normal haemoglobin A2 level: implication for population screening. Ann Clin Biochem. 2002; 39(1): 44-9.

doi: 10.1258/0004563021901720.

[9] Traeger-Synodinos J, Vrettou C, Sofocleous C, Zurlo M, Finotti A, Gambari R. Impact of α-globin gene expression and α-globin modifiers on the phenotype of β-thalassemia and other hemoglobinopathies: Implications for patient management. Int J Mol Sci. 2024; 25(6): 3400. doi: 10.3390/ijms25063400.

[10] Vijian D, Wan Ab Rahman WS, Ponnuraj KT, Zulkafli Z, Bahar R, Yasin N, et al. Gene mutation spectrum among alpha-Thalassaemia patients in Northeast Peninsular Malaysia. Diagnostics. 2023; 13(5): 894. doi: 10.3390/diagnostics13050894.

[11] Knappenberger JA, Kuriakose SA, Vu BC, Nothnagel HJ, Vuletich DA, Lecomte JTJ. Proximal influences in two-on-two globins: Effect of the Ala69Ser replacement on synechocystis sp. PCC 6803 hemoglobin. Biochemistry. 2006; 45(38): 11401-13. doi: 10.1021/bi060691x.

[12] Narayanan S, Mathew B, Srinivasu BY, Bhat V, Ross C, Mandal AK. Effect of point mutation on structure–function correlation of hemoglobin variants, HbE and HbD Punjab. Amino Acids. 2020; 52(6-7): 893-904. doi: 10.1007/s00726-020-02858-9.

[13] Pornprasert S, Ruengdit C, Punyamung M, Sripichai O. Alpha-Thalassemia caused by ααIVSI-1(AGGT> AGAT) (HBA1: c.95 + 1G > A) Mutation and its combinations with other forms of thalassemia or hemoglobinopathy in Northern Thailand. Indian J Hematol Blood Transfus. 2025; 41(3): 674-9. doi: 10.1007/s12288-024-01895-8.

[14] Panyasai S, Chantanaskulwong P, Permsripong N, Mokmued T. Interactions of electrophoretically silent hemoglobin Hekinan II [HBA1:c.84G>T] with various forms of α-thalassemias and other hemoglobinopathies: novel insights into the molecular and hematological characteristics and genetic origins. Libyan J Med. 2024; 19(1): 2406620. doi: 10.1080/19932820.2024.2406620.