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Case Report : Hemoglobin H disease caused by compound heterozygosity for α^0-thalassemia Lamphun deletion and α^+-thalassemia 3.7 kb deletion ($--^{Lamphun}/-\alpha^{3.7}$) in a Thai elderly case

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ABSTRACT

We report the deletional HbH disease caused by compound heterozygosity for α^0-thalassemia Lamphun type deletion and α^+-thalassemia 3.7 kb deletion ($--^{Lamphun}/-\alpha^{3.7}$) in a Thai elderly (61-year-old) subject. She was seen by her physician for fatigue and dyspnea. A physical examination revealed that she was a slightly pale patient. A complete blood cell count (CBC) showed microcytic hypochromic anemia (Hb 91 gm/L, MCV 73.0 fL, and MCH 21.0 pg). However, she had no history of receiving a blood transfusion. Thus, a better understanding of the genotype, phenotype, and hematological and clinical features of this HbH disease ($--^{Lamphun}/-\alpha^{3.7}$) will be helpful in treatment, genetic counseling, prevention, and control programs of thalassemia in Thailand.

Introduction

α-thalassemia is a hereditary blood disorder characterized by the impaired production of α-globin chains, vital hemoglobin components. This condition arises from mutations, especially the deletions of *HBA1* and *HBA2* genes located on chromosome 16. The α-globin chains are essential for the proper formation and function of hemoglobin (Hb), and their deficiency leads to a range of clinical manifestations, from mild anemia to severe, and life-threatening conditions. Clinically, α-thalassemia presents a spectrum of severity depending on the number and type of α-globin gene deletions.[1] The condition is classified into several forms, including α^+-thalassemia trait (-α/αα), α^0-thalassemia trait (--/αα), HbH disease (--/-α), and the most severe form, Hb Bart's hydrops fetalis (--/--). Each form presents unique clinical challenges and management needs. For instance, individuals with HbH disease may experience moderate to severe anemia and require regular monitoring and intervention. At the same time, those with Hb Bart's hydrops fetalis face a high risk of perinatal death without intrauterine interventions.[2]

The α^0-thalassemia Southeast Asian ($--^{SEA}$) deletion accounted for 98% of the Thai samples with HbH disease, while the α^0-thalassemia Thai ($--^{THAI}$) deletion made up the rest. Additionally, a 3.7 kb deletion accounted for 91% of α^+-thalassemia, with a 4.2 kb deletion comprising the remainder of the deletional HbH disease. Meanwhile, the

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Hb Constant Spring (CS, *HBA2*: c.427T>C) variant was the predominant form of non-deletional HbH disease.[3] To date, there are over 200 different mutations associated with α-thalassemia.[4] Recently, studies conducted in Thailand have demonstrated the use of next-generation sequencing (NGS) to identify a rare mutation essential for disease prevention and control. The α^0-thalassemia Chiang Rai ($--^{CR}$) deletion, a 44.6 kb deletion on chromosome 16, was observed in Hb Bart's hydrops fetalis in combination with $--^{SEA}$, thus completely removing both *HBA1* and *HBA2* genes.[5] Subsequently, this mutation was identified in several unrelated cases of deletional and non-deletional HbH diseases.[6] Moreover, a 27.0 kb deletion and a small insertion of 9 bp (CGGGAGGGA) within the α-globin gene cluster (NC_000016.10:g.158985_186051delinsCGGGAGGGA), named α^0-thalassemia Lamphun ($--^{Lamphun}$) deletion, was reported in a 2-year-old Thai boy diagnosed with uncommon deletional HbH disease ($--^{Lamphun}/-\alpha^{3.7}$). The causative α-thalassemia alleles were inherited from his mother ($--^{Lamphun}/\alpha\alpha$) and father ($-\alpha^{3.7}/\alpha\alpha$).[7]

Case Report

In this study, the authors report the discovery of deletional HbH disease caused by compound heterozygosity for $--^{Lamphun}/-\alpha^{3.7}$ in an elderly case. She is a 61-year-old Thai woman living in Lamphun, a province in northern Thailand, who a physician saw at a private hospital for fatigue and dyspnea. Upon examination, she appeared pale and did not have hepatosplenomegaly. In 2018, she was diagnosed with colon cancer and underwent 12 cycles of chemotherapy. However, she had never received a blood transfusion. A complete blood count (CBC) was analyzed using an automated blood counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan). Laboratory findings are as follows: RBC $4.3x10^{12}$ cells/L, Hb 91 gm/L, PCV 0.32 L/L, MCV 73.0 fL, MCH 21.0 pg, MCHC 29.0 gm/L, and RDW 20.5%. Due to the low levels of Hb, MCV, and MCH, combined with a high level of RDW, she was diagnosed with microcytic hypochromic anemia. However, iron deficiency anemia could be excluded because her serum iron (SI=135 μg/dL) and total iron-binding capacity (TIBC=188 μg/dL) were within normal values. In contrast, her serum ferritin (SF=2,865 μg/L) was higher than the normal range. Therefore, her blood sample was sent to the Associated Medical Sciences-Clinical Service Center (AMC-CSC), Chiang Mai University, Chiang Mai Province, Thailand, for thalassemia diagnosis. In the thalassemia laboratory, hemoglobin analysis was performed using capillary electrophoresis (CE, Capillarys 2 Flex Piercing, Sebia, Norcross, Georgia). The peaks of HbH (16.1%), Hb Bart's (0.6%), and a low level of HbA_2 (0.7%) were found on the CE electropherograms (Figure 1). Thus, deletional HbH disease was suspected, and molecular analysis for the diagnosing of α-thalassemia was performed. Genomic DNA was extracted from the blood sample using the NucleoSpin® kit (Macherey-Nagel, KG., Duren, Germany) following the manufacturer's instructions. A single-tube multiplex real-time PCR with EvaGreen and high-resolution melting (HRM) analysis for the diagnosis of α^0-thalassemia $--^{SEA}$, $--^{THAI}$, and $--^{CR}$ deletions was performed as described previously (8). Furthermore, as previously described, a multiplex gap-PCR was performed to detect α^+-thalassemia-$\alpha^{3.7}$ and -$\alpha^{4.2}$ kb deletions.[7] The single-tube multiplex real-time PCR with EvaGreen and HRM analysis showed a negative result for α^0-thalassemia $--^{SEA}$, $--^{THAI}$, and $--^{CR}$ deletions, while the multiplex gap-PCR revealed a positive result for α^+-thalassemia -$\alpha^{3.7}$ kb deletion. As described previously, conventional gap-PCR was further performed to detect α^0-thalassemia $--^{Lamphun}$ deletion.[7] The PCR product of approximately 1.2 kb, specific for α^0-thalassemia $--^{Lamphun}$ deletion, was revealed (Figure 2). Based on these results, she was diagnosed with deletional HbH disease caused by compound heterozygosity for α^0-thalassemia Lamphun deletion and α^+-thalassemia 3.7 kb deletion ($--^{Lamphun}/-\alpha^{3.7}$).

Name	%	Normal Values %
Hb H	16.1	
Hb Bart's	0.6	
Hb A	82.6	
Hb A2	0.7	

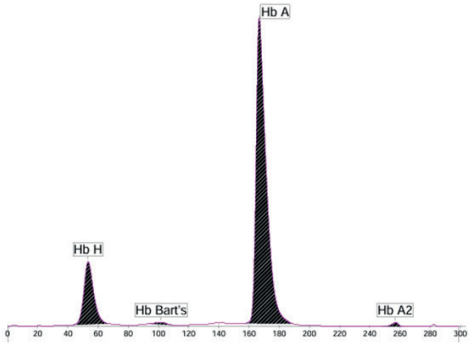

Figure 1. *The CE electropherogram of the case.*

Discussion

Nowadays, there are over 200 different mutations associated with α-thalassemia, and at least 20 types of α^0-thalassemia deletion have been described.[2,4] However, many deletions remain unknown and cannot be identified using conventional techniques. These unknown α-thalassemia deletions can pose a risk of misdiagnosis, leading to conditions such as Hb Bart's hydrops fetalis and HbH disease. In the previous study, we characterized a new 27.0 kb deletion with a small insertion of 9 bp (CGGGAGGGA) in the α-globin gene cluster, spanning both the *HBA1* and *HBA2* genes. This mutation was named α^0-thalassemia Lamphun ($--^{Lamphun}$) deletion based on the geographic origin of the first case.[7] This deletion is larger than α^0-thalassemia $--^{SEA}$ (19.3 kb) but smaller than the $--^{THAI}$ (33.4 kb) and $--^{CR}$ (44.6 kb) deletions, which are the most prevalent *cis* deletions found in the Thai population.[8] In the present study, we reported HbH disease resulting from compound heterozygosity for $--^{Lamphun}/-\alpha^{3.7}$ in a 61-year-old Thai woman. Although this elderly case and the previous case are from unrelated families, they live in Lamphun Province, a province in northern Thailand. Therefore, both may share the same ancestry. These two cases exhibited moderate anemia with a total Hb level of 91 gm/L; neither required blood transfusion. However, the increased serum ferritin in the subject could be explained by the pathophysiology of increased iron absorption from the gastrointestinal (GI) tract. The clinical and hematological features of the compound heterozygosity for $--^{Lamphun}/-\alpha^{3.7}$ are comparable to those of deletional HbH diseases caused by compound heterozygosity for $--^{SEA}/-\alpha^{3.7}$, $--^{THAI}/-\alpha^{3.7}$, and $--^{CR}/-\alpha^{3.7}$, and they are less severe than non-deletional HbH diseases.[9-11] Compound heterozygosity for α^0-thalassemia $--^{Lamphun}$ deletion and other Hb variants, including HbCS, Hb Paksé (*HBA2*: c.429A>T), Hb Pak Num Po (*HBA1*: c.396_397insT), and Hb Quong Sze (*HBA2*: c.377T>C), may result in severe non-deletional HbH disease. Therefore, a better understanding of these cases' clinical and hematological features, especially for the new and rare α^0-thalassemia mutation, such as the $--^{Lamphun}$ deletion and its combinations, is beneficial for management planning, genetic counseling, treatment, and family education.

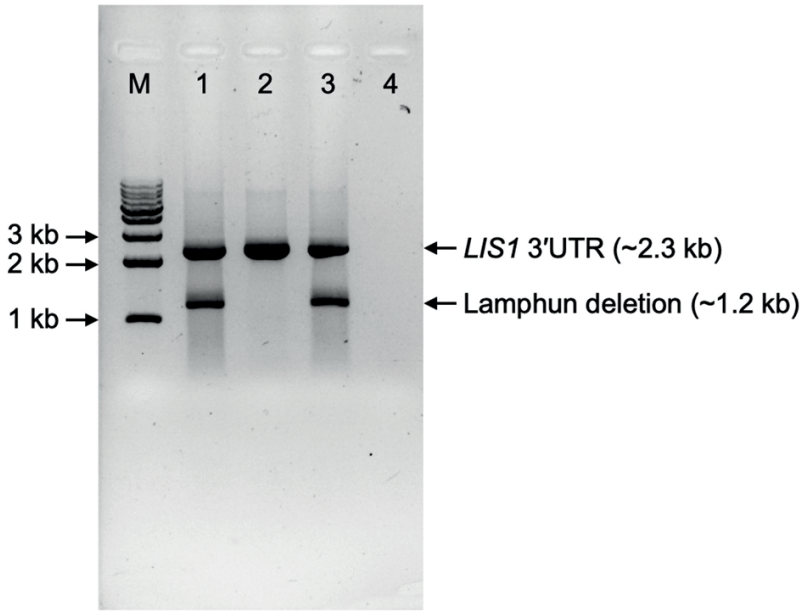

Figure 2. *Conventional gap-PCR identified the α^0-thalassemia $--^{Lamphun}$ deletion (~1.2 kb). The amplified fragments were separated by 1.0% agarose gel electrophoresis. M: 1 kb ladder DNA marker, Lanes 1, 2, 3, 4: the analysis results for the positive control, negative control, case, and no DNA template control, respectively. The amplification of the LIS1 3'UTR fragment (~2.3 kb) was used as an internal control.*

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Ethical approval

This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (AMSEC-67EM-037).

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Conflict of interest

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

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