Coinherited Hemoglobin H/Constant Spring Disease and Heterozygous Hemoglobin Tak Causing Severe Hemolytic Anemia in a Thai Boy

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Summary: Hemoglobin (Hb) H/Constant Spring disease is a common nondeletional Hb H disease, typically causing a more severe phenotype than the deletional Hb H disease counterpart. Hb Tak, resulting from a dinucleotide insertion (+AC) at codon 146 of beta-globin gene, has an increased oxygen affinity and usually presents with polycythemia. We studied a case of a 4-year-old Thai boy with a severe, early-onset anemia. To our knowledge, he is the first reported patient with Hb H/Constant Spring disease and heterozygous Hb Tak. Trio–whole-exome sequencing does not identify other genetic variants that may contribute to the severity of anemia. The observation suggests that coinherited Hb H/Constant Spring and heterozygous Hb Tak lead to severe hemolytic anemia.

Key Words: coinheritance, hemoglobin Constant Spring, hemoglobin H disease, hemoglobin Tak, hemolytic anemia

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Thalassemias and hemoglobin (Hb) variants are common hereditary red blood cell disorders in Southeast Asian countries including Thailand.[1] Although thalassemias are characterized by quantitative defects in the synthesis of the globin chain subunits, Hb variants result from the mutations, causing structural or functional defects. Hb Tak is a Hb variant that was initially reported in a Thai boy from Tak province in Thailand.[2] The variant is caused by a dinucleotide insertion (+AC, rs3999427; NM_000518.5: c.440_441dup,p.*148Thrext*11) into codon 146 of the beta-globin gene (*HBB*), causing a frameshift mutation leading to a chain elongation and increased oxygen affinity. The typical clinical manifestation of patients with beta-thalassemia/Hb Tak is polycythemia.[3–5] Hb Constant Spring is caused by a nucleotide substitution (rs41464951; NM_000517.4: c.427T > C, p.Ter143Gln, also known as p.Ter142Gln) at a termination codon of alpha 2-globin gene (*HBA2*) resulting in an elongated and unstable Hb variant. Hb H/Constant Spring, which is a nondeletional Hb H disease, is typically more severe than the deletional Hb H disease counterpart, with lower baseline Hb levels and higher proportion of patients needing regular transfusions in the long term.[6–8]

The coinheritance of Hb H disease and *HBB* mutations are not uncommon.[9,10] However, only 4 cases with deletional Hb H disease and heterozygous Hb Tak have been reported.[9,10] All cases had mild-to-moderate anemia. To the best of our knowledge, our report is the first description of Hb H/Constant Spring and a coinherited heterozygous Hb Tak. The combination results in an early-onset severe hemolytic anemia. Whole-exome sequencing analysis showed no other mutations that might have added to the severity of anemia.

CASE REPORT

A 4-year-old Thai boy was referred from a provincial hospital to our institute for a definite diagnosis of thalassemia disease and iron chelation plan. His parents were northern Thais. The 24-year-old father was diagnosed with Hb H disease. He never received transfusion. The 20-year-old healthy mother was known from prenatal screening to be a carrier of an unspecified Hb variant. There was no history of congenital anemia in the extended family members. The patient was born at term. He had neonatal indirect hyperbilirubinemia, which was treated with 7 days of phototherapy. When the patient was 1-month old, his parents noticed that he was pale and took him to the hospital. The initial physical examination showed marked pallor and enlarged liver and spleen. Initial investigations revealed microcytic anemia. He was diagnosed with Hb H disease by Hb analysis. Blood transfusion was started at 1 month of age and continued every 3 to 4 weeks. At 4 years of age, his steady-state pretransfusion Hb levels were 8.4 to 9.7 g/dL. However, the Hb levels decreased to 6 to 7 g/dL when he had fever. His weight and height were at the 10th and 25th to 50th percentile, respectively. After a total of 13 transfusions, when the patient was 15 months old, he had iron overload with ferritin level of 1645 ng/mL. He received an iron chelator for iron overload.

At our institute, the investigations were performed to identify the type of thalassemia disease. The complete blood count was carried out by using the Sysmex XS-800i hematology analyzer (Sysmex, Kobe, Japan). The Hb analysis was carried out by high-pressure liquid column chromatography using the Variant II high-pressure liquid column chromatography system (Bio-Rad Laboratories, CA) according to the manufacturer's recommendation. The complete blood count and Hb analysis results from the patient are presented in Table 1. The patient had microcytic anemia. The Hb analysis result from the patient's posttransfusion sample showed AA_2 pattern and 1.6% abnormal Hb at the retention time of 4.23 minutes. The father had a typical Hb AHBart's pattern of Hb H disease. The Hb analysis from the mother showed AA_2, A_2 2.5% and 23.9% abnormal Hb at the retention time of 4.13 minutes. Common *HBA* mutations including Southeast Asian, Thai, −3.7 kb, −4.2 kb deletions, Hb Constant Spring, and Hb Pakse mutations were screened for by polymerase chain reaction–based methods. Common *HBB* mutations were screened for by polymerase chain reaction and high-resolution melting analysis, as previously

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TABLE 1. Characteristic Features of Reported Cases With Hb H Disease and Heterozygous Hb Tak

	This Report	Fucharoen and Fucharoen[9]	Panyasai et al[10]	Panyasai et al[10]	Panyasai et al[10]
Age, sex	4 y, male	10 y, male	Unknown	Unknown	Unknown
Hb (g/dL)	8.4*	8.8	8.9	6.6	10.1
MCV (fL)	68.2	66.9	51.3	60.5	57.8
MCH (pg)	21.9	19.1	16.3	18.8	18.3
MCHC (g/dL)	32.1	28.5	—	—	—
RBC ($\times 10^{12}$/L)	3.84	4.6	5.7	3.5	5.5
RDW (%)	28.2	36.4	—	—	—
Hb analysis	AA_2Tak A_2 3.0% Tak 1.6%*	AA_2Tak A_2 2.9% Tak 7.8%	A_2 3.7% F 4.9% Tak 6.2%	A_2 1.9% F 33.4% Tak 6.7%	A_2/E 50.1% F 5.9% Tak 30.9%
Hb H inclusions	Not done	None	Not done	Not done	Not done
Genotype	$--^{SEA}/\alpha^{CS}\alpha$, $\beta;/\beta^{Tak}$	$--^{SEA}/-\alpha^{-3.7}$, β/β^{Tak}	$--^{SEA}/-\alpha^{-3.7}$, β/β^{Tak}	$--^{SEA}/-\alpha^{-3.7}$, β/β^{Tak}	$--^{SEA}/-\alpha^{-3.7}$, β^{E}/β^{Tak}
Diagnosis	Hb H/Constant Spring disease and Hb Tak	Hb H disease/Hb Tak	Hb H disease/Hb Tak	Hb H disease/Hb Tak	Hb H disease/Hb E and Hb Tak
Clinical features	Early-onset of anemia, transfusion-dependent	Mild anemia, no hepatosplenomegaly, no history of blood transfusions	NA	NA	NA

*Blood count and Hb analysis results at 4 years of age between monthly transfusions. The sample was taken 4 weeks after the previous transfusion.

Hb indicates hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NA, not applicable; RBC, red blood cell count; RDW, red cell distribution width.

described.[11] The *HBB* mutation detected by high-resolution melting analysis was further confirmed by an automated DNA sequencing.[12] The molecular identification revealed that the patient had Hb H/Constant Spring disease (Southeast Asian deletion/*HBA2* codon 142 (T > C), $--^{SEA}/\alpha^{CS}\alpha$) with heterozygous Hb Tak (*HBB* codon 146 (+AC), β/β^{Tak}). The father had deletional Hb H disease (Southeast Asian and 3.7 kb deletions, $--^{SEA}/-\alpha^{-3.7}$). The mother was a carrier of both Hb Constant Spring ($\alpha\alpha/\alpha^{CS}\alpha$) and Hb Tak ($\beta/\beta^{Tak}$).

To search for other genes that may add to the severity of anemia, trio–whole-exome sequencing analyses from the patient and both parents were performed, as per the method described previously.[13] The analysis did not reveal other genes that may result in anemia.

DISCUSSION

This case report suggests that the coinheritance of Hb H/Constant Spring and heterozygous Hb Tak results in severe hemolytic anemia. The hematologic manifestation is more severe than those seen in cases with deletional Hb H disease and heterozygous Hb Tak in the previous reports.[9,10] The effects of other genes on the severity of anemia are not found by whole-exome sequencing analysis.

Hb H disease is caused by mutations of 3 of 4 alleles of *HBA*, leaving one intact allele. The disease can be classified into deletional and nondeletional Hb H disease according to the type of mutations on the alpha$^+$-thalassemia allele. Patients with nondeletional Hb H disease usually have more severe hemolytic anemia than those with deletional Hb H disease.[6–8] Among nondeletional Hb H diseases, Hb H/Constant Spring disease is the most common genotype.[1] Hb Constant Spring is caused by a point mutation from T > C at codon 142 or termination codon, resulting in an amino acid substitution and an elongation of alpha-globin. A significant minor fraction of patients with Hb H/Constant Spring disease may require transfusion in the long term.[7,8]

The pathophysiology of Hb Constant Spring involves several mechanisms. Hb Constant Spring carriers typically have a low level of ~1% Hb Constant Spring.[14] The low level of Hb Constant Spring has been shown to be related to the instability of alpha-Constant Spring mRNA.[15–17] A study of alpha-globin mRNA expression in a subject with Hb H/Hb Constant Spring by Liebhaber and Kan[15] reveals that alpha-Constant Spring mRNA is present in erythroid precursors from the bone marrow but absent in reticulocytes. A study in transgenic mouse lines also reveals that a selective degradation of alpha-Constant Spring mRNA occurs early in bone marrow erythroid cells but not late in peripheral reticulocytes.[16] The mRNA destabilization is paralleled by the phased shortening of the poly(A) tails. A study in murine erythroleukemia cells suggests that the mutation at the termination codon of *HBA2* allows a translational read through of ribosome into the 3′ untranslated region, resulting in a disruption of a determinant that is required for mRNA stability in erythroid cells.[17]

Patients with Hb H/Constant Spring typically have more severe disease than those with deletional Hb H disease. Moreover, individuals with homozygous Hb Constant Spring have moderate anemia and elevated Hb Bart, unlike the carrier phenotype in homozygous deletional alpha-plus thalassemia. A study in homozygous Hb Constant Spring shows that, in addition to mRNA instability and a decreased alpha-globin protein synthesis as a mechanism of anemia, the cessation of globin protein synthesis and the destruction of excess beta-globin chains occurs unusually rapidly.[18] Schrier and colleagues demonstrates that there are both oxidized beta-globin chains and oxidized alpha-Constant Spring globin chains on the red cell membranes of Hb Constant Spring red cells. The oxidized globin chains cause a damage to red cells and are likely the cause of the more severe phenotype in Hb H/Constant Spring than in deletional Hb H disease.[6]

Approximately 20% to 30% of the Thai population are carriers of alpha-thalassemia.[1] In the northern Thai population, the estimated prevalence of deletional and nondeletional Hb H disease is 13.6:1000 and 1.6:1000,

respectively.[19] Coinheritance of Hb H disease with other Hb variants is common. The coinheritance with Hb E resulting in AEBart's or AEBart's Constant Spring disease is the most common and well-studied entity.[20,21] Homozygous Hb Constant Spring, although generally characterized by mild hemolytic anemia, has been reported to associate with fetal anemia and hydrops, which can be improved with intrauterine transfusion.[22] The interaction between the alpha-Constant Spring and gamma-globin is possibly the cause of severe anemia in the intrauterine period that improved after birth.

Hb variants in Thailand are common. In one large cohort study, among 26,013 normal subjects investigated, 636 people (2.4%) were found to carry Hb variants.[16] Hb Tak was one of the most common beta-globin chain variants that comprised 14.2% of all Hb variants detected.[10,23] Hb Tak is caused by a dinucleotide insertion (+AC) between codon 146 and the termination codon 147. This causes a frameshift mutation that results in a substitution of the C-terminal histidine residue by threonine and an elongation of beta-globin peptide. The mutated region functions in the conformational changes of the Hb molecule between fully oxygenated and deoxygenated Hb. Hb Tak mutation results in a high oxygen affinity, noncooperativity, and lack of Bohr effect.[3,4,24] Individuals with heterozygous Hb Tak (β/β^{Tak}) are usually asymptomatic with normal Hb level or borderline polycythemia.[10,25] Compound heterozygosity of beta-thalassemia and Hb Tak (β^0/β^{Tak}) and Hb E/Hb Tak (β^E/β^{Tak}) typically result in polycythemia rather than in anemia.[4,5,10] Homozygous Hb Tak has been reported with polycythemia and complications of hyperviscosity.[4]

Previous reports of 4 cases with Hb H disease and Hb Tak are reviewed in Table 1. All cases have deletional Hb H disease ($--^{SEA}/-\alpha^{-3.7}$). Three have heterozygous Hb Tak, and 1 has compound heterozygosity of Hb E and Hb Tak (β^E/β^{Tak}). One case of a 10-year-old boy with Hb Tak and Hb H disease has mild anemia, no hepatosplenomegaly, and no history of blood transfusion.[9] The other 2 cases with Hb Tak and Hb H disease and 1 case with Hb Tak/Hb E and Hb H disease have mild-to-moderate anemia, although the clinical information is not available.[10] The case with Hb H/Constant Spring and Hb Tak in our study has more severe disease. This could be from the interaction between the alpha-Constant Spring globin and beta-Tak globin that increases the degree of hemolysis. Of note, in one previously reported case with deletional Hb H disease/Hb Tak, the Hb H and Hb Bart and Hb H inclusion bodies were absent. This may be explained by the decreased levels of normal beta-globin in Hb Tak carriers. In our case, the Hb analysis was performed after several transfusions, and hence we could not conclude whether the Hb H and Hb Bart were absent or not. Apart from the severe anemia requiring transfusion, it was also observed that the patient had an early onset of iron overload. The iron loading could be from both transfusions and the increased iron absorption associated with ineffective erythropoiesis.

Our case illustrates a complex genotype of Hb H/Constant Spring and heterozygous Hb Tak resulting in an increased severity of hemolytic anemia. In the regions with high prevalence of thalassemia and Hb variants, coinheritance causing complex genotypes is frequently encountered. Thorough molecular characterization is essential to establish a correct diagnosis for treatment planning and genetic counseling.

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REFERENCES

1. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia. *Hemoglobin*. 1987;11:65–88.
2. Flatz G, Kinderlerer JL, Kilmartin JV, et al. Haemoglobin Tak: a variant with additional residues at the end of the beta-chains. *Lancet*. 1971;1:732–733.
3. Imai K, Lehmann H. The oxygen affinity of haemoglobin Tak, a variant with an elongated beta chain. *Biochim Biophys Acta*. 1975;412:288–294.
4. Tanphaichitr VS, Viprakasit V, Veerakul G, et al. Homozygous hemoglobin Tak causes symptomatic secondary polycythemia in a Thai boy. *J Pediatr Hematol Oncol*. 2003; 25:261–265.
5. Charoenkwan P, Thanarattanakorn P, Chaovaluksakul S, et al. Hematological and molecular characterization of beta-thalassemia/Hb Tak compound heterozygote. *Southeast Asian J Trop Med Public Health*. 2003;34:415–419.
6. Schrier SL, Bunyaratvej A, Khuhapinant A, et al. The unusual pathobiology of hemoglobin constant spring red blood cells. *Blood*. 1997;89:1762–1769.
7. Lal A, Goldrich ML, Haines DA, et al. Heterogeneity of hemoglobin H disease in childhood. *N Engl J Med*. 2011;364:710–718.
8. Charoenkwan P, Taweephon R, Sae-Tung R, et al. Molecular and clinical features of Hb H disease in northern Thailand. *Hemoglobin*. 2005;29:133–140.
9. Fucharoen S, Fucharoen G. Hb H disease with various beta hemoglobinopathies: molecular, hematological and diagnostic aspects. *Hemoglobin*. 2012;36:18–24.
10. Panyasai S, Fucharoen G, Fucharoen S. Hemoglobin variants in Northern Thailand: prevalence, heterogeneity and molecular characteristics. *Genet Test Mol Biomarkers*. 2016;20:37–43.
11. Charoenkwan P, Sirichotiyakul S, Phusua A, et al. High-resolution melting analysis for prenatal diagnosis of beta-thalassemia in northern Thailand. *Int J Hematol*. 2017;106:757–764.
12. Sirichotiyakul S, Saetung R, Sanguansermsri T. Analysis of beta-thalassemia mutations in northern Thailand using an automated fluorescence DNA sequencing technique. *Hemoglobin*. 2003; 27:89–95.
13. Ittiwut C, Natesirinilkul R, Tongprasert F, Sathitsamitphong L, et al. Novel mutations in *SPTA1* and *SPTB* identified by whole exome sequencing in eight Thai families with hereditary pyropoikilocytosis presenting with severe fetal and neonatal anaemia. *Br J Haematol*. 2019;185:578–582.
14. Weatherall DJ, Clegg JB. The alpha-chain-termination mutants and their relation to the alpha-thalassaemias. *Philos Trans R Soc Lond B Biol Sci*. 1975;271:411–455.
15. Liebhaber SA, Kan YW. Differentiation of the mRNA transcripts originating from the alpha 1- and alpha 2-globin loci in normals and alpha-thalassemics. *J Clin Invest*. 1981;68:439–446.
16. Morales J, Russell JE, Liebhaber SA. Destabilization of human alpha-globin mRNA by translation anti-termination is controlled during erythroid differentiation and is paralleled by phased shortening of the poly(A) tail. *J Biol Chem*. 1997;272: 6607–6613.
17. Weiss IM, Liebhaber SA. Erythroid cell-specific determinants of alpha-globin mRNA stability. *Mol Cell Biol*. 1994;14: 8123–8132.
18. Derry S, Wood WG, Pippard M, et al. Hematologic and biosynthetic studies in homozygous hemoglobin Constant Spring. *J Clin Invest*. 1984;73:1673–1682.
19. Lemmens-Zygulska M, Eigel A, Helbig B, et al. Prevalence of alpha-thalassemias in northern Thailand. *Hum Genet*. 1996;98: 345–347.

20. Chaibunruang A, Karnpean R, Fucharoen G, et al. Genetic heterogeneity of hemoglobin AEBart's disease: a large cohort data from a single referral center in northeast Thailand. *Blood Cells Mol Dis*. 2014;52:176–180.
21. Boonsa S, Sanchaisuriya K, Fucharoen G, et al. The diverse molecular basis and hematological features of Hb H and AEBart's diseases in Northeast Thailand. *Acta Haematol*. 2004;111:149–154.
22. Sirilert S, Charoenkwan P, Sirichotiyakul S, et al. Prenatal diagnosis and management of homozygous hemoglobin constant spring disease. *J Perinatol*. 2019;39:927–933.
23. Srivorakun H, Singha K, Fucharoen G, et al. A large cohort of hemoglobin variants in Thailand: molecular epidemiological study and diagnostic consideration. *PLoS One*. 2014;9: e108365.
24. Imai K, Tientadakul P, Opartkiattikul N, et al. Detection of haemoglobin variants and inference of their functional properties using complete oxygen dissociation curve measurements. *Br J Haematol*. 2001;112:483–487.
25. Shih MC, Wu KH, Liu SC, et al. Hb Tak: a beta chain elongation at the end of the beta chain, in a Taiwanese. *Hemoglobin*. 2005;29:65–67.